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Direct liquid chromatographic determination of hydrazines: A review

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ABSTRACT

Today the determination of hydrazines is an important application in analytical chemistry. This review shows the current state-of-the-art analyses and discusses the merits of the direct chromatographic methods for the determination of hydrazines such as ion-, ion-exclusion, ion-pair and hydrophilic interaction chromatography. The methodological aspects of the separation and detection of hydrazines are considered for these methods. Examples of hydrazine determination in real samples are presented.

1. Introduction

Hydrazines are hazardous chemicals widely used in the laboratory and industry. Hydrazine (Hy) and its methylated analogs such as methylhydrazine (MH) and 1,1-dimethylhydrazine (UDMH) are employed as high-energy rocket propellants. The problem of the environmental pollution by rocket fuel and its decomposition components is of special significance for Russia, Kazakhstan, China and other countries [1], where hydrazines are still widely used for the rocket launches and polluted sites that originate from the unburned rocket fuel dispersed after the fall of the rocket stages.

Hydrazine is commonly added to boiler feed water for the deoxygenation and corrosion protection in the power plants. Due to its reducing properties it is also used in metal plating, semiconductor processing and as an antioxidant [2,3].

Hydrazines have many applications in the synthesis of various pesticides, polymers, foaming agents, drugs, etc. [2,3], and they can remain as impurities in these industrial products. Furthermore, hydrazines may appear as hydrolytic products of agrochemicals in the environment and food [4,5].

Hydrazines are toxic agents that may induce mutagenesis, carcinogenesis and internal organ injuries [6]. Thus, in order to prevent the unwanted intakes in humans, analytical techniques should be used to control hydrazines in the environment, industrial products, food, and pharmaceuticals. Being the metabolites of some pharmaceuticals [7–10], hydrazines should be determined simultaneously with the other metabolites in the biofluids for evaluating the drug efficiency.

This article reviews various analytical approaches for the determination of hydrazines without derivatization based upon the application of ion, ion-pair, ion-exclusion and hydrophilic interaction chromatography. It focuses especially on the methodological issues and shows the potential advantages of the reported analytical methods.

2. Discussion

Determination of hydrazine and its alkylsubstitued analogs is challenging because of their high polarity, tendency to oxidize, absence of chromophores and their low molecular weight. Chromatographic methods are superior to other analytical methods due to their selectivity and reliability; most detection methods used with chromatography are sensitive as well. Most known chromatographic techniques developed for hydrazines require pre-column derivatization. However, these derivatization techniques are labor-intensive and suffer from drawbacks related to time-consuming reactions, and the presence of reagent artifacts, or unwanted derivatization by-products which can hinder analyte detection. In some cases, the derivatization process leads to the low reproducibility owing to incomplete reaction and poor stability of the derivatization products.

Being polar N-containing compounds, hydrazines interact with fused silica of the capillary columns, which results in the strong peak tailing and complicate their direct determination by gas chromatography (GC). Liquid chromatography (LC) is a more suitable separation technique for the direct determination of hydrazines, though not all methods of liquid chromatography are equally successful in the separation of hydrazines (Table 1).

Reversed phase retention of hydrazine and methylhydrazines was demonstrated for standard solutions in some reports [11–14]. However, peak efficiency was lower than usually obtained for non-polar compounds, which is not surprising due to the known interactions between N-containing bases and silanol groups. Polar hydrazines show weak retention on the hydrophobic reversed phases, therefore peak overlap between hydrazines and other unretained substances should be expected

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Table 1
Experimental details for analytical methods used to determine hydrazines, aliphatic amines and hydroxylamine.

Method	Analyte	Sample type	Technique details	Remarks	LOD	Reference
RPLC–AD	Hy MH SDMH UDMH	Standard solution	ODS column; 0.1 M KNO_3+0.01 M phosphate buffer, pH 7; electrochemically pretreated glassy carbone electrode (GCE), E=0.5 V	Low resolution of Hy/MH and SDMH/UDMH peaks; conditions for enhancement of AD response of hydrazines were proposed	4.7 μg/L 5 μg/L 150 μg/L 39 μg/L	[11]
FIA–AD	Hy	Standard solution	0.20 M NaOH; carbon paste electrode modified by cobalt phthalocyanine, $E=-0.1$ V	Unsuitable for RPLC mobile phase; enhancement of AD response	0.32 μg/L	[12]
RPLC–AD	Hy MH SDMH	Standard solution	μBondapak C18 (250 mm × 4 mm); 0.1 M phosphate buffer, pH 7.0; GCE modified by Vitamin B-12, E=0.45 V	Low resolution of Hy/MH peaks; enhancement of AD response	16 μg/L 46 μg/L 90 μg/L	[13]
RPLC–AD	Hy MH SDMH	Standard solution	μBondapak C18 (250 mm × 4 mm); 0.1 M · phosphate buffer, pH 7.0; GCE coated with an electrodeposited film of mixed-valent cobalt oxide/cyanocobaltate, E=0.8 V	Low resolution of Hy/MH peaks; enhancement of AD response	4.8 μg/L 14 μg/L 30 μg/L	[14]
IC–CD	Methylamine Ethylamine Dimethylamine Trimethylamine	Scrubbing solution	IonPac CS 10 (250 mm × 4 mm), 40 mM methanesulfonic acid (MSA) spiked with 10^{-7} M Na^+	Method was applied for determination of aliphatic amines in the atmosphere	0.5 μg/L[a]	[19]
IC–CD	Amylamine tert-Butylamine	Pharmaceuticals	IonPac CS 10 (250 mm × 4 mm), MSA–MeCN	Sample was dissolved in water and directly injected into the IC system	(0.01%)[b]	[20]
IC–CD, IC–AD	Ethanolamine Dimethylethanolamine Triethanolamine Tetramethylammonium	Pore liquid expressed from cement paste	IonPac $CS1_4$ (250 mm × 4 mm); H_2SO_4 or MSA–MeCN	Application for determination of corrosion inhibitors for steel reinforcement in concrete	5 (CD), 0.5 mM (AD) 8 mM (CD) 0.5 mM (AD) 3 mM (CD)	[22]
IC–CD	mono-, di-, tri-and tetra-substituted methyl- and ethylamines	Standard solution	Silasorb SPH-C18 (50 mm × 3 mm) modified by dodecylbenzenesulfonic acid; 5 mM HCl –0.1 mM dodecylbenzenesulfonic acid;	Simultaneous separation of alkali metals and amines (up to 5 analytes)	10 μg/L for low-molecular-mass amines	[23]
IC–CD	Ethanolamines, C1-C4 mono-, di-, tri-and tetra-substituted alkylamines, cyclohexylamine	Standard solution	Waters IC-Pak Cation M/D (150 mm × 4.6 mm); HNO_3–0.1 mM EDTA–MeOH	Simultaneous separation of alkali metals and amines (up to 10 analytes)	25 μg/L for low-molecular-mass amines	[24]
IC–CD	Methylamine Dimethylamine Trimethylamine	Standard solution	Vydac 400 IC (50 mm × 4.6 mm); 4 mM sulfamic acid	Sulfamic acids as eluent in ion chromatography for determination of NH_4^+, alkali ions and alkyl amines was evaluated	200 μg/L 500 μg/L 1000 μg/L	[25]
IC–CD	Methylamine Ethylamine Trimethylamine Trimethylamine-N-oxide	Standard solution	Metrosep C2 (250 mm × 4 mm); 3 mM HNO_3—3.5% (v/v) MeCN	Application for organic nitrogen aerosol analysis was demonstrated	43 μg/L 46 μg/L 76 μg/L 72 μg/L	[26]
IC–CD	Hy	Pharmaceuticals	Metrosep C2 (150 mm × 4.6 mm); 5 mM HNO_3—10% (v/v) acetone	Sample was dissolved in eluent and directly injected into the IC system	1530 μg/L	[28]
IC–PD	Hy Hydroxylamine	Standard solution	Nucleosil 10 SA (300 mm × 4 mm); 2 mM citrate and 2 mM ethylenediamine, pH 4.5	Hy overlapping with the system peak	300 μg/L 300 μg/L	[29]
IC–AD	UDMH SDMH MH Hy	Standard solution	Aminex A-5 (300 mm × 4 mm); 0.05 M sodium borate buffer, pH 8.9; E=0.8 V	Hydrazines were not fully protonated in separation condition	80 μg/L[a] 100 μg/L[a] 170 μg/L[a] 800 μg/L	[30]
IC–AD	Hy	Pharmaceuticals	IonPac CS14 (250 mm × 4 mm); 10 mM $HClO_4$; E=0.8 V	Sample was dissolved in methanol–water (50:50,	25 μg/L (0.005%)[b]	[31]

Table 1 (*continued*)

Method	Analyte	Sample type	Technique details	Remarks	LOD	Reference
				v/v) and directly injected into the IC system		
IC–AD	Hydroxylamine	Waste streams	IonPac CS14 (250 mm × 4 mm); 11 mM H_2SO_4; post-column addition of NaOH; pulsed mode for AD	Sample dilution 1:10	1.5 μg/L (0.015 ppm)[b]	[32]
IC–AD IC–CD	Hydroxylamine Methylhydroxylamine N,N-Dimethylhydroxylamine	Standard solution	Alltech/Wescan Cation/R (150 mm × 4.6 mm); 10 mM HNO_3; post-column addition of NaOH for AD; E=0.8 V	Interference of Na^+ for hydroxylamine determination eliminates by using AD	330 μg/L (CD), 6.6 μg/L (AD) 1140 μg/L (CD), 57 μg/L (AD) 1420 μg/L (CD), 114 μg/L (AD)	[33]
IC–AD	Hy MH SDMH UDMH	Standard solution	Nucleosil 10 SA (100 mm × 4 mm); 50 mM ammonium phosphate buffer, pH 6.9; E=1.0 V	The selectivity of silica cation-exchangers with respect to separation of amines and hydrazines was investigated	0.4 μg/L 0.6 μg/L 2 μg/L 1 μg/L	[34]
IC–AD	Hy MH UDMH	Standard solution	Nucleosil 10 SA (100 mm × 4 mm); 50 mM ammonium acetate buffer, pH 5.3; E=1.2 V	Application for soil analysis	0.2 μg/L 0.5 μg/L 1 μg/L (0.08 mg/kg)[a,b]	[36]
IC–AD	Hy, MH, Et-Hy SDMH, UDMH 1-Me-1-Et-Hy, tert-Bu-Hy 1,2-diEt-Hy, triMe-Hy Bu-Hy TMT	Standard solution	Nucleosil 10 SA (150 mm × 4 mm); 30 mM ammonium acetate buffer, pH 5.3; E=1.2 V	Simultaneous separation of hydrazines (up to 7 analytes)	0.2, 0.5, 1.2 μg/L 1.8, 0.8 μg/L 3, 8 μg/L 7, 12 μg/L 3, 5 μg/L	[37]
IPC–AD	Hy, MH, Et-Hy SDMH, UDMH 1-Me-1-Et-Hy, tert-Bu-Hy 1,2-diEt-Hy, triMe-Hy Bu-Hy TMT	Standard solution	Diasphere 110-C16 (150 mm × 4 mm); 1 mM odium octylsulfonate, 75 mM ammonium acetate buffer, pH 3.9, 1% (v/v) MeCN; E=1.2 V	Simultaneous separation of all listed hydrazines	0.4, 0.9, 3.6 μg/L 1.3, 0.4 μg/L 2, 90 μg/L 11, 0.2 μg/L 70, 5 μg/L	[37]
GC–MS	UDMH	Soil	DB-5 (30 m × 0.32 mm); SIM, m/z=193, 226	Aniline as internal standard; derivatization with 4-nitrobenzaldehyde, LLE	(0.01 mg/kg)[b]	[38]
GC–NPD GC–MS	UDMH	Soil	DB-5 (30 m × 0.32 mm); SIM, m/z =193, 226	Aniline as internal standard; derivatization with 4-nitrobenzaldehyde, LLE	(0.003 mg/kg)[b] (0.008 mg/kg)[b]	[39]
TLC–FLD	Hy	Pharmaceuticals	Kieselgel 60 RP2 silanized; water–MeOH (50:50, v/v); densitometric scanning in the fluorescent mode, λ_{em}=366 nm	Sample dissolution, derivatisation with salicylaldehyde, LLE; for carbidopa analysis pre-separation on anion-exchanger was required	(1 ppm)[b]	[40]
RPLC–UV	Hy	Pharmaceuticals	Altima C18 (250 mm × 4.6 mm), 0.03% EDTA–MeCN (30:70, v/v); λ=305 nm	Sample dissolution, derivatisation with benzaldehyde, LLE; for carbidopa analysis pre-separation on anion-exchanger was required	10 μg/L (0.2 ppm)[a,b]	[40]
IC–AD	Hy	Pharmaceuticals	IonPac CS14 (250 mm × 4 mm); 10 mM $HClO_4$; E=0.8 V	Advantages of IC–AD for determination of Hy in pharmaceuticals was demonstrated	25 μg/L[a] (0.8 ppm)[a,b]	[40]
IC–AD	UDMH	Natural water	Separation column: Nucleosil 10 SA (100 mm × 4 mm); concentrator column: Nucleosil 10 SA (50 mm × 4 mm); 50 mM ammonium acetate buffer, pH 5.4; E=1.2 V	Sample distillation for removing of interfering cations; on-line preconcentration of a 10 mL sample	0.1 μg/L	[41]
IC–AD	UDMH	Standard solution	Separation column: Nucleosil 10 SA (250 mm × 4 mm);	On-line preconcentration of a 100 mL sample; method	0.02 μg/L	[42]

Table 1 (*continued*)

Method	Analyte	Sample type	Technique details	Remarks	LOD	Reference
			concentrator column: Nucleosil 10 SA (50 mm × 4 mm); 100 mM ammonium acetate buffer, pH 5.4; E=1.2 V	can be used to samples with low mineralization only		
IC–UV–AD	NDMA Hy MH UDMH TMT	Standard solution	Synergi Hydro RP (150 mm × 4 mm), 5 mM sodium octylsulfate or 0.05 mM sodium dodecylsulfate, 200 mM ammonium acetate buffer, pH 4.4, 8.5-10% (v/v) MeCN; λ=240 nm, E=1.2 V	Simultaneous determination of NDMA and hydrazines	7 (UV) μg/L 0.3 (AD) μg/L 0.7 (AD) μg/L 0.8 (AD) μg/L 1.2 (AD), 20 (UV) μg/L	[43]
IEC–CD	Hy	Standard solution	Separation column: TSKgel DEAE-5 PW; water; enhancement columns: TSKgel SAX in the SO_4^{2-}-form and TSKgel SCX in the H^+-form	Application of IEC–CD for determination of Hy in boiler water was demonstrated	0.64 μg/L	[45]
IEC–UV	Hy	Natural water, boiler water	Separation column: TSKgel DEAE-5 PW; water; enhancement column: TSKgel SAX in the I^--form; λ=230 nm	Na^+, NH_4^+ were well separated from Hy and do not interfere for its determination	0.26 μg/L	[46]
HILIC–CLND	Hy UDMH	Standard solution	ZIC HILIC (250 mm × 4.6 mm); TFA/water/ethyl alcohol (0.1:30:70, v/v/v)	Factors affecting the separation of hydrazines in the HILIC were studied; application for analysis of spiked samples of pharmaceuticals was demonstrated	2000 μg/L (0.02%)[a,b] (S/N=9) 2000 μg/L (0.02%)[a,b] (S/N=28)	[47]
HILIC-MS–MS-ESI	Trimethylammonium,1,1,1-trimethylhydrazinium, 3-hydroxy-1,1-dimethyl-4,5-dihydro-1H-pirazolium-1-betaine, 3-(2,2,2-trimethylhydrazinium)methylpropionate, 3-(2,2,2-trimethylhydrazinium)ethylpropionate, 3-(2,2,2-trimethylhydrazinium)prop-2-yl-propionate	Pharmaceuticals	ZIC HILIC (100 mm × 2.1 mm); 5 mM ammonium formate- MeCN, pH 5.0 (15:85, v/v).	Factors affecting the separation of mildronate and its impurities in the HILIC were studied	(0.01%)[a,b], (0.001%)[a,b], (0.002%)[a,b], (0.001%)[a,b], (0.0004)[a,b], (0.0002%)[a,b]	[48,49]

AD—amperometric detection, CD—conductometric detection, PD—potentiometric detection, CLND—chemiluminescent nitrogen detection

[a] LOQ

[b] respect to sample

when real samples are analyzed by reversed phase LC. Therefore, other modes of separation should be considered. Hydrazines are Bronsted bases and they are protonated in acidic solutions, which allows ion analysis methods to be applied for the direct determination of hydrazines.

2.1. Ion chromatography

Ion chromatography (IC) was first introduced in 1975 [15] and since that time the technique has grown in usage for ions and ionizable compounds at a surprising rate because it provided a simple, reliable, and inexpensive method for the simultaneous multi-component determination in complex mixtures [16,17]. Ion chromatography has become one of the main powerful analytical tools for the analysis of complex matrices and speciation studies in the field of metal analysis [18]. Nowadays, the range of solutes that can be determined by IC continues to expand, mainly more organic ionic species can now be determined.

Short-chain aliphatic amines were successfully determined by the most popular IC methods with conductivity detection in the direct [19–22] and indirect [23–27] modes using the same separation conditions as for inorganic cations. The selectivity of separation columns was sufficient for separating both metals and amines, therefore, non-selective conductivity detection was used. A similar strategy can be applied for the separation of hydrazines, which are bases like amines, but it is rarely used. Only a single application [28] was published for the determination of hydrazine in the "Cilazapril" drug, where conductivity detection followed the separation of hydrazine from the active pharmaceutical ingredient on a Metrosep C2 column by eluting with a 5.0 mM nitric acid and acetone mixture (90: 10 v/v). The limit of detection (LOD) was 1.5 mg/L. Low sensitivity of a conductivity detector requires the alternative schemes for detecting hydrazines.

For investigating the applicability of potentiometric detection of the reducing species with the metallic copper electrode, hydrazine and hydroxylamine were separated on the Nucleosil

10SA cation-exchange column (SCX-type silica with sulfobenzoic functional groups) with 2 mM citrate–2 mM ethylenediamine (pH 4.5) as a mobile phase [29]. The chromatogram was complex with Hy overlapping with the system peak. The LOD for hydroxylamine was 10 nmol corresponding to the 0.1 mL injection of 3.3 mg/L of hydroxylamine.

Fiala and Kulakis [30] were the first to introduce the technique for the determination of hydrazines based on IC with amperometric detection (IC–AD). Hy, MH, UDMH and 1,2-dimethylhydrazine (SDMH) were separated within 25 min on a Aminex A-5 column packed with a high capacity polymer cation-exchanger with sulfonic acid type functional groups. The capacity of this column being too high, the appropriate retention times were achieved using the mobile phase with pH 8.9 where hydrazines were not fully protonated. The retention order of hydrazines was UDMH < SDMH < MH < Hy and correlated with the degrees of protonation of the separated species in a borate buffer (pK_a values for Hy, MH, SDMH and UDMH were 8.07, 7.87, 7.52, and 7.21, respectively). A glassy carbon electrode was operated at +1.0 V (vs. Ag/AgCl). The limits of quantitation (LOQ) were 17 (0.17), 10 (0.1), and 8 ng (0.08 mg/L) for MH, SDMH, and UDMH respectively using an injection volume of 0.1 mL. The Hy peak was obscured by the noise at the levels below 80 ng (0.8 mg/L). These concentration parameters were rather high due to the low efficiency of the selected separation system. But even in this case, the amperometric detector was more sensitive than the conductometric detector.

A direct IC method on a polymeric IonPac CS-14 cation column using amperometric detection in a direct current (DC) mode with a platinum electrode (potential +0.8 V) is described for the determination of hydrazine in the intermediate for a drug synthesis with a LOD of 25 μg/L [31]. The mobile phase was 10 mM perchloric acid. The application of this or other mineral acid solution as a background electrolyte for the amperometric detection of hydrazines does not seem to be a very good choice as the amperometric response of hydrazines decreases with increasing pH value. In similar methods for the determination of hydroxylamines (hydroxylamine and its N-alkylderivatives) [32,33], based upon separation on a cation-exchanger column with mineral acid eluents, the post-column addition of a strong base was used to achieve the desired pH value ~10. The LOD value for hydroxylamine with a DC amperometric detection on pretreated glassy carbon electrodes at 0.6 V was 6.6 μg/L with an injection volume of 0.05 mL [33]. An application of pulsed amperometric detection on a gold electrode with the same injection volume provides LOD of 1.5 μg/L hydroxylamine [32].

Smolenkov et al. focused on the development of IC–AD for the determination of hydrazines on silica-based stationary phases which exhibit higher separation efficiency in comparison with polymer-based ones. Both silicas with chemically bonded sulfonic acid groups and reversed phase silicas dynamically coated with dodecylbenzenesulfonic acid were used for the separation of hydrazines [34]. Initially the selectivity of cation-exchangers in the separation of Li^+, Na^+, NH_4^+, K^+, hydroxylamine, Hy, MH, SDMH, UDMH and C_1–C_3 aliphatic amines with methanesulfonic acid as a mobile phase was investigated using ion chromatography with conductivity detection. Although the selectivities of cation-exchangers differed from each other, this complex mixture was not completely separated due to the close retention of hydrazines and amines with the similar structures. Furthermore, one essential feature should be noted. SDMH was retained less than UDMH for sulfonic acid bonded silica; the opposite behavior was exhibited by reversed phase silicas modified with dodecylbenzenesulfonic acid.

A broader list of hydrazines and aliphatic amines was further investigated by IC with mass-spectrometric detection [35]. Two columns (Nucleosil SA and Diasorb SA) were studied with ammonium acetate buffer as an eluent. The selectivity for the separation of hydrazines was found to coincide with the pattern of the retention of known organic ions, increasing with the number of carbon atoms in the alkyl substituent chain and with the number of substituents in the molecule. It was also established that doubly substituted symmetrical hydrazines are retained less than unsymmetrical ones. A comparison of ethyl- and butylhydrazine retention times with those of disubstituted methyl- and ethyl-hydrazines, respectively, showed a weaker retention for ethylhydrazine in comparison with 1,1- and 1,2-dimethylhydrazines. For butylhydrazine, the limited water solubility and consequent disruption of water structure begins to act, resulting in its stronger retention as compared to 1,2-diethylhydrazine.

A comparison of the retention characteristics of hydrazines and corresponding amines demonstrated the weaker retention of hydrazines. Ethylhydrazine was retained more weakly than ethylamine, but stronger than methylamine. A similar behavior was observed for butylhydrazine, which retention time lies between those of propyl- and butylamine. At the same time, the decrease in the retention times of disubstituted methyl- and ethylhydrazines is not large enough to determine the homologous amine with a smaller number of substituents, i.e., methylamine and ethylamine, respectively.

Amperometric detection with glassy-carbon working electrode using acetate and phosphate buffers as mobile phases was next proposed for the determination of hydrazines [34,36]. The best sensitivity for Hy, MH and UDMH was achieved with mobile phases in the pH range of 5.3–5.9 due to the better separation efficiency and higher peaks, in spite of the competing reaction of protonation decreasing the peak areas in acidic solutions. The potential value of +1.2 V was selected when voltamperograms achieved a plateau where the maximum amount of substances was oxidized. The LODs of IC-AD in aqueous solutions using 0.25 mL sample volume were 0.2, 0.5, and 1 μg/L for Hy, MH and UDMH, respectively.

The retention order of hydrazines on Nucleosil SA using an ammonium acetate buffer as eluent was Hy < MH < Et-Hy ≈ SDMH < UDMH < 1-Me-1-Et-Hy ≈ tert-Bu-Hy < 1,2-diEt-Hy ≈ triMe-Hy < Bu-Hy « tetramethyl-2-tetrazene (TMT) [37]. The selectivity of separating the group of hydrazines with intermediate retention is modest, and no more than 7 species can be separated simultaneously (Fig. 1); but this is enough for solving

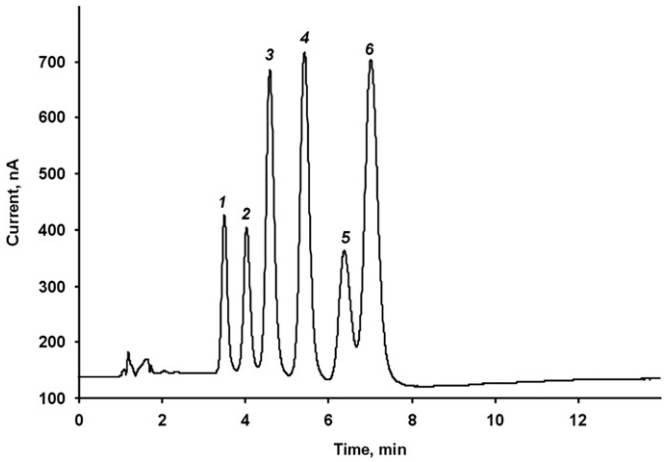

Fig. 1. IC separation of hydrazines. Column: Nucleosil 10 SA (100 mm × 4.0 mm). Mobile phase: 75 mM ammonium acetate buffer solution (pH 5.3). Amperometric detection, +1.2 V. 1, Hy; 2, MH; 3, ethylhydrazine; 4, UDMH; 5, tert-butylhydrazine; and 6, butylhydrazine.

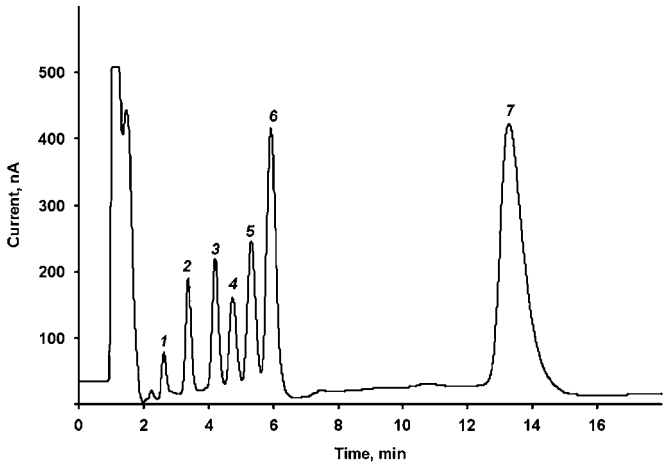

Fig. 2. IC separation of N-containing compounds. Column: Nucleosil 10 SA (100 mm × 4.0 mm). Mobile phase: 100 mM ammonium acetate buffer solution (pH 5.3), 20% acetonitrile. Amperometric detection, +1.2 V. 1, unknown; 2, hydroxylamine; 3, Hy; 4, MH; 5, SDMH; 6, UDMH; and 7, TMT.

the practical task of the simultaneous determination of UDMH and such products of its degradation as Hy, MH and TMT (Fig. 2).

Applications of IC–AD for various real samples demonstrate that this method proves fast and convenient analysis along with sufficient reliability and good sensitivity. High specificity of IC–AD is guaranteed by the shorter list of compounds that can be retained on cation-exchangers and oxidized; this is in contrast to HPLC-UV usually applied for the determination of organic substances. Moreover, the well-known character of the regularities of ion-exchange makes it possible to anticipate and to investigate the behavior of probable interfering substances in the selected chromatographic and detection conditions. For example, as methyl- and dimethyl-amines can interfere with the determination of UDMH according to the data on the separation selectivity, their interference was studied. No peak was observed for 10 mM methylamine solution with amperometric detection. Dimethylamine could interfere if its concentration was more than 1 mM.

A rapid method for the determination of 0.08–40 μg/kg UDMH in soils was proposed [36]. IC–AD was sensitive enough to determine UDMH at the Russian Federation maximum permissible concentration level of 0.1 μg/kg. The method included the distillation of UDMH from an alkaline soil suspension followed by analysis of the distillate by IC. Being sensitive enough, the IC approach requires neither pre-derivatization nor liquid–liquid extraction which burdens GC-analysis [38,39].

The determination of hydrazine in pharmaceutical substances and drugs may be recommended in accordance with the results reported by Kean et al. [40]. They demonstrated the concordance of a direct IC–AD method (3.9 mg/kg) with an approved TLC method (3.2 mg/kg) and HPLC-UV (3.0 mg/kg). IC examination, as opposed to TLC and HPLC, was performed directly after sample dissolution in water. Other methods are based upon a derivatization procedure, and additional clean-up on anion exchange column is necessary prior to the derivatization for eliminating matrix effects.

Apart from other advantages, IC makes it possible to implement a simple and automatic on-line pre-concentration technique in order to improve the sensitivity significantly. High concentration factors, no concentrate dilution, and use of the treated sample in full are characteristic features for IC coupling with on-line pre-concentration. The most common approach for the on-line pre-concentration of hydrazines involves replacing the injection loop with a microcolumn filled with the same cation-exchanger as the one used in the separation column. Preconcentration of a 100 mL sample aliquot decreased the LOD of UDMH to 0.02 μg/L, sufficient for determining UDMH at the Russian Federation maximum permissible concentration level of 0.06 μg/L for drinking water [41]. Matrix cations in real samples at high ionic strengths interfere. To eliminate the influence of metal cations, the sample was made alkaline and distilled over to an acetic acid absorber. This combined distillation-based technique followed by the analysis of 10 mL of the distillate using IC–AD with on-line concentration gives LODs of 0.003, 0.06 and 0.1 μg/L for Hy, MH and UDMH, respectively [42].

2.2. Ion-pair chromatography

In ion-pair chromatography (IPC), the ionic nature of the sample is suppressed by the association with an ion-pair reagent of the opposite charge. The resulting uncharged ion-pair interacts with a non-polar stationary phase. So, IPC is an alternative to IC for the separation of ionic species not only on the basis of their retention mechanism but also with a fundamentally different separation selectivity as demonstrated by the separation of aliphatic hydrazines and tetramethyl-2-tetrazene (Fig. 3). In comparison with IC, IPC showed enhanced selectivity and improved resolution [37], which allowed a more complex mixture containing 11 components to be separated. Thus, IPC can successfully compete with IC for the separation of hydrazines.

Another distinction from IC is that IPC can be used for the simultaneous determination of compounds of both ionogenic and nonionogenic nature. For this reason, IPC may be considered as a technique for the simultaneous determination of UDMH and its decomposition products such as Hy, MH, TMT and nitrosodimetylamine (NDMA). The last is a neutral compound but it was retained on the hydrophobic surface of alkylsilica. The separation of the mixture mentioned above by IPC was thoroughly studied [43,44].

Ion-pair chromatography is very versatile and offers more possibilities for changing the stationary and/or mobile phase parameters. Among the mobile phase parameters controlling the retention of the ion-pair are polarity, ionic strength and pH of the mobile phase, and the size, concentration and lipophilic nature of the counter-ion. It was shown that the addition of C_7–C_9 alkylsulfonates to a mobile phase at the concentrations over the range of ~1–10 mM can provide the separation [44]. Since decreasing the length of the alkyl chain from C_9 to C_7 leads to a decrease in the retention and lower resolution, it can be balanced by decreasing the buffer concentration in the eluent or increasing

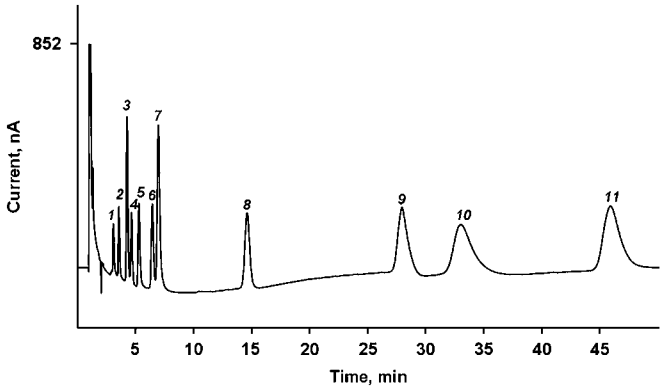

Fig. 3. IPC separation of hydrazines. Column: Diaspher C16 (5 μm, 150 mm × 4.60 mm). Mobile phase: 75 mM ammonium acetate buffer solution (pH 4.4), 1% acetonitrile and 0.05 mM of sodium octylsulfonate. Amperometric detection, +1.2 V. 1, Hy; 2, MH; 3, UDMH; 4, SDMH; 5, ethylhydrazine; 6, methylethylhydrazine; 7, trimethylhydrazine; 8, 1,2–diethylhydrazine; 9, tert-butylhydrazine; 10, TMT; and 11, butylhydrazine.

the ion-pair reagent concentration. The retention time of the last component of the system, TMT, depends to a large degree on the organic modifier content in the eluent. The addition of 8%–10% acetonitrile to the eluent reduces the analysis time to 11 min. However, it is the minimum possible time of chromatographic run because further increasing the acetonitrile content dramatically reduces the retention of slightly hydrophobic NDMA.

Sodium octylsulfonate, octylsulfate, and dodecylsulfate were compared as ion-pair reagents [43]. Using octylsulfate instead of octylsulfonate resulted in the higher retention (two times higher retention factor for TMT) and good selectivity towards the separation of all ionogenic components as compared to using octylsulfonate. As 0.05 mM sodium dodecylsulfate showed the same separation as 5 mM octylsulfate, using the former would be preffered; the reagent consumption is much less and the reagent is widely available.

The separation of Hy, MH, UDMH, TMT and NDMA is shown in Fig. 4. NDMA contains no electroactive groups, therefore, spectrophotometric detection was additionally used at 240 nm. The LODs 0.3, 0.7, 0.8, 7 and 1.2 μg/L were achieved for Hy, MH, UDMH, NDMA and TMT, respectively for a 0.1 mL sample volume.

2.3. Ion-exclusion chromatography

As weak bases, hydrazines can also be separated by ion-exclusion chromatography (IEC), but the attempt to use this method was performed only for Hy [45,46]. Hydrazine was determined in boiler waters including the separation from ammonium ion, alkali and alkaline earth metal cations. It is clear that metal cations are not retained in IEC mode, and hydrazine can be successfully separated from the matrix. The separation of ammonium and hydrazine was performed using a Tosoh TSKgel DEAE-5PW weakly basic anion-exchange resin column (7.5 mm × 150 mm) with water as an eluent.

The amperometric detector cannot operate with pure water as a background medium due to its low conductivity; the lack of sensitive detector is a drawback of the proposed separation system. To achieve ppb level detection of hydrazine by ion-exclusion chromatography, the amplification of the hydrazine conductivity signal was proposed. It utilized two ion-exchange enhancement columns connected in series with the outlet of the separation column [45]. In the first anion-exchange column in SO_4^{2-}-form, the exchange reaction of converting N_2H_5OH into $(N_2H_5)_2SO_4$ took place. Then in the second cation-exchange column in H^{+-}-form, $(N_2H_5)_2SO_4$ was converted into H_2SO_4. The use of two enhancement columns provided a 6.10-fold and 26.8-fold enhancement in the detector responses for ammonium and hydrazine, respectively. The LOD of Hy was 0.64 μg/L with the sample volume of 0.1 mL. The analysis of boiler water could be accomplished within 8 min.

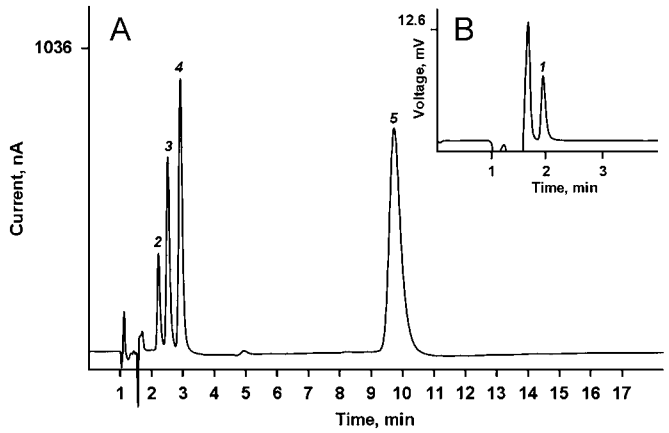

Fig. 4. IPC separation of UDMH and its decomposition products. Column: Synergi Hydro RP (4 mm × 150 mm). Mobile phase: 200 mM ammonium acetate buffer solution (pH 4.4), 8.5% acetonitrile and 5 mM of sodium octylsulfate. Amperometric (A) and UV- (B) detection, E=1.2 V, λ=240 nm. 1, NDMA; 2, Hy; 3, MH; 4, UDMH; and 5, TMT.

The same principle of increasing the sensitivity was used in another study [46], with the TSKgel SAX anion-exchange column in I^--form following the separating column. The compounds obtained were detected with a UV-detector at 230 nm by the absorption of I^-. That approach provided a decrease in the LOD of hydrazine to 0.26 μg/L, which is comparable with amperometric detection.

2.4. Hydrophilic interaction chromatography

Hydrophilic interaction chromatography (HILIC) is a valuable technique which is complementary to other chromatographic methods for the determination of highly hydrophilic and polar compounds. Though direct methods based on the ionic nature of hydrazines seem to be enough for solving the task of the determination of hydrazines, HILIC has certain advantages that are worthy of exploration.

First of all, HILIC is more useful in the analysis of drugs, active pharmaceutical ingredients, and their synthetic intermediates which have hydrophobic nature and are soluble in organic solvents only. Organic solvents are weak solvents in HILIC separations and their high content in the sample diluents is suitable for the HILIC mode due to the elimination of time-consuming and labor-intensive sample preparation for the solvent reconstitution required for other chromatographic methods.

Before the method development, the separation mechanism of hydrazines was studied on ZIC-HILIC column with zwitterionic sulfobetaine groups and alcohols which are rarely used as eluents in HILIC [47]. The retention times of analytes were observed to decrease with the increasing polarity of the mobile phase gained by water content and the type of organic solvent. The retention of positively charged hydrazines decreased with an increase of the ionic strength. These effects were not unexpected due to the fact that both electrostatic and hydrophilic interactions contributed to the retention and separation of the hydrazines. At the same time, some phenomena connected with the strong influence of the nature of the added buffer or acid on the retention are difficult for theoretical explanation.

The separation of SDMH, UDMH, MH and Hy was achieved within 15 min using TFA/water/ethyl alcohol (0.1/30/70, v/v/v). HILIC shows the retention order opposite to IPC: SDMH < UDMH < MH < Hy, which is not surprising due to the increasing native polarity.

The optimized HILIC method coupled with chemiluminescent nitrogen detection was applied to the simultaneous determination of hydrazine and 1,1-dimethylhydrazine in the hydrophobic pharmaceutical intermediate after the sample dilution in DMSO/ethyl alcohol (30/70, v/v) mixture. The method is simple and sensitive enough with an LOQ of 0.02%.

The other reason for choosing HILIC is a widespread combination of the properties of analytes, when their high polarity prohibits a reversed-phase mode and UV-detection is not suitable because of the lack of distinguished chromophores, which makes mass spectrometry (MS) the appropriate detection alternative. HILIC is more suitable for mass-spectrometric detection in comparison with IPC where the addition of ion-pair agents reduces the sensitivity of mass-spectrometric detection. At the same time, high organic solvent content in the eluent applied in HILIC has a favorable influence on the sensitivity of ESI-MS-detection.

The application of HILIC-MS–MS-ESI is illustrated for the determination of six impurities in the active pharmaceutical ingredient mildronate (3-(2,2,2-trimethylhydrazinium)propionate

dihydrate) [48,49] including cations such as trimethylammonium and 1,1,1-trimethylhydrazinium, zwitterions (mildronate itself and 3-hydroxy-1,1-dimethyl-4,5-dihydro-1H-pirazolium-1-betaine) and methyl-, ethyl- and isopropyl- esters of mildronate with cationic nature. Special attention was given to the influence of the mobile phase composition on the retention of the analytes from the complex mixture mentioned above and separation selectivity in the HILIC mode on the several different polar stationary phases (silica, cyano-, amino- and the zwitterionic sulfobetaine). It was shown that the variation of the acetonitrile concentration is effective for adjusting the retention but this does not influence the selectivity significantly. The pH and buffer concentrations are of prime importance for adjusting the retention of cations and cationic esters, but have little effect on zwitterions.

For a complete separation of all components in a mixture of mildronate and its impurities (although this is not essential in MS detection) the following chromatographic conditions were selected: zwitterionic sulfobetaine stationary phase (ZIC-HILIC) with acetonitrile/5 mM ammonium formate with pH 5.0 (85/15%, v/v) as a mobile phase. Under such conditions, mildronate is the most retained component and analysis takes 50 min.

The LOQs of impurities were 0.01% of the assay or lower; while preliminary quality specifications require a $\leq 0.1\%$ content. The proposed method showed good linearity ($r^2 > 0.99$) and precision (RSD from 2.1% to 9.3%) over the tested range of 0.05%–0.125%. The method was applied to two batches of mildronate. In the technical batch, the levels of residual trimethylammonium and 1,1,1-trimethylhydrazinium bromides were 0.20 and 0.35%, respectively. But in the commercial batch, 0.01% of trimethylammonium bromide was found. 1,1,1-Trimethylhydrazinium bromide was at the level (0.0003%) mentioned as the LOD of the method. Thus, HILIC–MS method seems to be useful for the simultaneous determination of hydrazines and their analogs.

3. Conclusions

For rugged and reliable results in routine analysis, minimum effort and low cost solutions are required. Nowadays, methods based upon the derivatization techniques for the determination of hydrazines still prevail despite the derivatization leading to an overall long analysis time and introducing many imprecision contributions.

It is clear from the body of the work reviewed above that direct methods for the determination of hydrazines offer significant operational advantages in terms of simplifying sample preparation and lowering analysis time. Modern direct chromatographic techniques have an excellent separation power and a wide range of orthogonal methods from IC to HILIC allows one to choose the optimal scheme for both separation of the components and sample preparation. Moreover, the amperometric detection sensitivity, applicable in this case, can reach sub-ppb level. It should also be noted that the task of the simultaneous determination of the substances of different classes can be solved using direct analysis methods, while difficulties of choosing a versatile reagent make this practically impossible for derivatization methods. These features of the direct chromatographic techniques for determination of hydrazines should be given wider recognition and such techniques should be made the official methods.

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