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Precise gas chromatography with retention time locking in comprehensive toxicological screening for drugs in blood

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Abstract

The long-term precision of three retention parameters, the absolute retention time (RT), the relative retention time related to dibenzepin (RRT), and the internal retention index based on the alkylfluoroaniline series (RI), were studied with 14 basic drugs on HP-5 and DB-17 columns with and without the use of the retention time locking option (RTL). Using the constant flow mode in all experiments, the RTL method was found to produce superior precision with all three retention parameters compared to the non-RTL method on each column. The results showed that RTL offers a significant advantage within a single instrument method, not only between methods, with CV<0.1% by RRT. Consequently, a dual-column gas chromatographic procedure with nitrogen–phosphorus detection was described for comprehensive screening for basic drugs in 1-ml whole blood samples. The method consisted of one-step liquid–liquid extraction with butyl acetate, identification using RRT in the RTL mode, and quantification based on single point calibration. The method allowed reliable screening and quantification of 124 basic drugs at therapeutic and toxic concentration levels in autopsy blood.

Keywords: Retention time locking; Toxicological screening

1. Introduction

The success of substance monitoring by chromatography in forensic, clinical, industrial and environmental applications depends largely on the precision of the retention parameter used with substance libraries. In gas chromatography (GC), retention index techniques have proved to be the most feasible solution for managing large libraries, especially on an interlaboratory basis [1,2], while the relative retention time is routinely used with in-house libraries of limited size. The absolute retention time is considered rather useless for library applications which only use chromatographic techniques. However, many users of gas chromatography–mass spectrometry (GC–MS) are content with the absolute retention time when they mainly rely on spectral information.

Recently, the concepts of method translation and retention time locking (RTL) in GC were introduced by Blumberg and Klee [3]. The idea is based on the fact that the void time is a universal time unit in GC, and method translation is the scaling of the time axis of the temperature programme relative to the void

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time. Method translation can be used for RTL, which allows chromatograms to be reproduced accurately from one GC to another or during a long period of time [4]. RTL has been successfully applied to multiresidue screening of pesticides in fruit and vegetable extracts by matching GC and GC–MS retention times to a common database [5].

A few years ago, the authors developed a series of dual-column GC screening methods for acidic [6] and basic drugs [7] and benzodiazepines [8] in the blood, using specially formulated retention index standards and dedicated software. The present study evaluates the impact of the novel RTL option on the basic drug screening by comparing the long-term precision of various retention parameters with and without using RTL.

2. Experimental

2.1. Materials

N,N-Dialkyl-4-fluoroanilines (FA Series) were prepared by alkylating 4-fluoroaniline as described elsewhere [9]. The drug substances were obtained from various pharmaceutical companies. Bovine whole blood was used for method development and calibration, and case blood samples were obtained at autopsy.

2.2. Data processing

The GC was operated and the data were collected, integrated and saved in ''txt'' format by ChemStation (Rev. A.08.03 Agilent Technologies, Palo Alto, CA, USA) software equipped with Retention Time Locking software (Rev. B.01.01). The data processing was carried out by SC Chrombooster software (Sunicom, Helsinki, Finland).

2.3. Gas chromatography

The GC was a 6890 Series plus instrument (Agilent Technologies) with two nitrogen–phosphorus detectors (NPD). The detectors (330 °C) were equipped with a capillary only extended jet (Agilent Technologies part number G1534-80580) and a small I.D. NPD collector funnel (G1534-20660) to minimize the tailing of peaks due to the geometry of the detector. The detector gas flows were 3 ml/min for hydrogen, 60 ml/min for air and 10 ml/min for nitrogen. The fused-silica capillary columns were HP-5 and DB-17 (15 m×0.32 mm I.D., 0.25 μm film thickness, Agilent Technologies). Uncoated deactivated fused-silica precolumns of 10 m×0.32 mm (Agilent Technologies) were connected to the analytical columns. The precolumns entered a single injector (270 °C) through a Graphpak™ 2M dual column injector adapter (Gerstel, Mülheim an der Ruhr, Germany). A deactivated straight liner (990 μl) with silanized glass wool was used in the injector. Automated injections were carried out with a 7683 Series injector (Agilent Technologies) using a 2 μl apparent injection volume.

The carrier gas was helium operated in the constant flow mode. The oven temperature was initially held at 100 °C for 0.4 min and then increased by 25 °C/min to 200 °C, then increased by 10 °C/min to 240 °C and then increased by 25 °C/min to 290 °C, where it was held for 10 min. The carrier gas flow was 2 ml/min for 15 min and then increased by 2 ml/min^2 to 4 ml/min, which was held for 6.4 min. Under these conditions with new analytical columns and new 10 m precolumns, the retention time of dibenzepin was 9.2 min on HP-5. This setting was locked by using five-point calibration data obtained with the nominal initial pressure and with pressures of −20%, −10%, +10% and +20% from the nominal initial pressure. Relocking based on one scouting run was carried out daily.

2.4. Sample preparation

Whole blood (1 ml) was transferred to a centrifuge tube (10 mm I.D.), Tris-buffer (1 *M*, pH 11, 0.3 ml) and the internal standard (dibenzepin 20 μg/ml in MeOH, 50 μl) were added (extraction pH 9.2), and the mixture was shaken. The sample was extracted with butyl acetate (0.3 ml) in a vortexer for 2 min and centrifuged, and an aliquot of the organic phase (150 μl) was transferred to an autosampler vial [9]. In the RI experiments, an aliquot of a solution containing the RI standards in butyl acetate was added to each vial prior to injection.

2.5. Limit of quantitation

The EURACHEM approach [10] with 20% precision was used for the calculation of the limit of quantitation (LOQ). In this approach, samples containing decreasing amounts of the analyte are injected six times. The calculated relative standard deviation is plotted against the analyte amount, and the amount that corresponds to the previously defined required precision is equal to LOQ.

3. Results and discussion

Tables 1 and 2 compare the long-term precision obtained using three different retention parameters on HP-5 and DB-17 columns, respectively. The retention parameters studied were the absolute retention time (RT), the relative retention time (RRT) related to dibenzepin, and the internal retention index based on the alkylfluoroaniline series (RI) [9]. The carrier gas programme was set at the constant flow mode in all experiments to facilitate the analysis of late eluting compounds, and the measurements were carried out without and with RTL. The drug substances represented various secondary and tertiary aliphatic amine structures. All results are based on 128 repetitive runs of spiked bovine blood extracts during an 18-week period.

Table 1
Precision (CV%) of the retention parameters on a HP-5 column without and with RTL

Compound	Without RTL			With RTL		
	RT	RRT	RI	RT	RRT	RI
Dibenzepin[a]	0.76		0.12	0.06		0.02
Fluoxetine	0.99	0.24	0.06	0.09	0.07	0.08
Tramadol	0.97	0.22	0.05	0.08	0.03	0.02
Metoprolol	1.03	0.30	0.15	0.12	0.10	0.09
Methadone	0.98	0.23	0.09	0.08	0.04	0.04
Nortriptyline	0.93	0.18	0.07	0.09	0.05	0.05
Mirtazapine	0.94	0.33	0.04	0.09	0.05	0.02
Maprotiline	0.87	0.12	0.11	0.09	0.06	0.05
Codeine	0.88	0.12	0.15	0.08	0.03	0.02
Citalopram	0.84	0.08	0.14	0.07	0.02	0.02
Chlorpromazine	0.65	0.11	0.06	0.08	0.05	0.03
Olanzapine	0.47	0.29	0.05	0.05	0.03	0.02
Hydroxyzine	0.47	0.29	0.07	0.05	0.04	0.05
Haloperidol	0.58	0.28	0.27	0.18	0.17	0.23
Thioridazine	0.63	0.18	0.12	0.14	0.11	0.08
Mean	0.80	0.21	0.10	0.09	0.06	0.05
Median	0.87	0.23	0.09	0.08	0.05	0.04

[a] Internal standard.

Table 2
Precision (CV%) of the retention parameters on a DB-17 column without and with RTL

Compound	Without RTL			With RTL		
	RT	RRT	RI	RT	RRT	RI
Dibenzepin[a]	0.60		0.12	0.12		0.13
Fluoxetine	1.05	0.45	0.16	0.11	0.06	0.16
Tramadol	1.13	0.53	0.29	0.16	0.05	0.20
Metoprolol	1.08	0.48	0.25	0.14	0.07	0.19
Methadone	1.10	0.50	0.20	0.18	0.06	0.14
Nortriptyline	0.94	0.33	0.19	0.15	0.04	0.13
Mirtazapine	0.82	0.23	0.16	0.13	0.03	0.13
Maprotiline	0.71	0.12	0.12	0.10	0.03	0.11
Codeine	0.67	0.06	0.13	0.12	0.01	0.13
Citalopram	0.63	0.05	0.09	0.08	0.05	0.10
Chlorpromazine	0.56	0.05	0.08	0.06	0.07	0.09
Olanzapine	0.86	0.26	0.43	0.17	0.05	0.29
Hydroxyzine	0.80	0.22	0.35	0.12	0.13	0.18
Haloperidol	0.36	0.20	0.22	0.12	0.06	0.05
Thioridazine	1.25	0.65	0.42	0.26	0.19	0.09
Mean	0.84	0.30	0.21	0.13	0.06	0.14
Median	0.82	0.25	0.19	0.12	0.06	0.13

[a] Internal standard.

There was a clear improvement in the precision (CV%) of all the three retention parameters on each column using the RTL function, the benefit being largest with RT and smallest with RI. All the RTL-based retention parameters showed very high precision without large mutual differences, however, RRT with an average CV below 0.1% on each column was in general the most precise approach for drug screening. The positive results obtained by RTL suggest that there was no column selectivity change which caused chromatographic variation, due to loading with biological extracts, as this could not be efficiently compensated by RTL.

Table 3 shows the relative retention times and linearity of quantitation for 124 basic drugs and metabolites on HP-5 and DB-17. In addition, the lower limits of quantitation (LOQ) obtained by single point calibration are listed. This selection of medicines includes most psychotropic and other

Table 3
Retention and quantification data for 124 basic drugs

Compound	RRT HP−5	RRT DB−17	Range (mg/l)[a] studied	R^2		LOQ (mg/l)[b]	Calibration point (mg/l)
				HP-5	DB-17		
Amitriptyline	0.800	0.797	0.1–3.0	0.9996	0.9995	0.1	0.5
Amphetamine[c]	0.216	0.211	0.5–5.0	0.9921	0.9956	0.5	2.0
Biperiden	0.859	0.836	0.1–3.0	0.9998	0.9998	0.1	0.5
Bisprolol	0.904	0.860	0.5–5.0	0.9117	0.8075	1.0	2.0
Brompheniramine	0.734	0.737	0.05–3.0	0.9996	0.9997	0.05	0.5
Bupivacaine	0.865	0.841	0.2–10	0.9986	0.9989	0.2	5.0
Buspirone	1.464	1.615	0.05–3.0	0.9978	0.9978	0.05	0.5
Caffeine	0.562	0.629	0.5–100	0.9953	0.9967	0.5	15
Carbamazepine[d]	0.894	0.985	1.0–50	0.9979	0.9965	1.0	15
Chlordiazepoxide[d]	1.045	1.054	0.2–10	0.9923	0.9925	0.2	3.0
Chloroquine	1.094	0.990	0.1–3.0	0.9987	0.9981	0.2	1.0
Chlorpheniramine	0.667	0.660	0.05–3.0	0.9996	0.9993	0.05	0.5
Chlorpromazine	1.028	0.981	0.05–3.0	0.9999	0.9999	0.05	1.0
Chlorprothixene	1.030	0.943	0.1–3.0	0.9992	0.9988	0.1	0.5
Cinchocaine	1.145	1.040	0.1–3.0	0.9996	1.0000	0.1	0.5
Cinnarizine	1.330	1.312	0.05–3.0	0.9957	0.9976	0.05	0.5
Citalopram	0.961	0.922	0.1–3.0	0.9999	0.9999	0.1	0.5
Clobutinol	0.530	0.490	0.2–5.0	0.9997	0.9979	0.2	2.0
Clomipramine	0.966	0.926	0.1–3.0	0.9960	0.9959	0.1	0.8
Clozapine	1.221	1.235	0.1–3.0	0.9951	0.9984	0.1	1.5
Cocaine	0.806	0.848	0.1–3.0	1.0000	1.0000	0.1	0.5
Codeine	0.943	0.966	0.1–3.0	0.9964	0.9989	0.1	0.5
Cyclizine	0.680	0.679	0.1–3.0	0.9996	0.9991	0.1	0.5
Dextrometorphan	0.757	0.757	0.1–3.0	0.9993	0.9993	0.1	0.5
Dextropropoxyphene	0.792	0.763	0.1–3.0	0.9992	0.9994	0.1	1.0
Diacetylmorphine	1.114	1.026	0.1–3.0	0.9999	0.9987	0.1	0.5
Diazepam	0.990	0.997	0.1–5.0	0.9995	0.9982	0.1	1.5
Dibenzepin[e]	1.000	1.000					
Diltiazem	1.261	1.314	0.1–3.0	0.9997	0.9993	0.1	0.5
Diphenhydramine	0.584	0.573	0.1–3.0	0.9991	0.9989	0.1	2.0
Disopyramide	1.035	1.000	0.2–20	0.9977	0.9431	0.2	5.0
Doxapram	1.230	1.219	0.1–3.0	0.9963	0.9963	0.1	2.0
Doxepin	0.825	0.837	0.05–3.0	0.9997	0.9995	0.05	0.5
Ethylmorphine	0.971	0.973	0.1–3.0	0.9996	0.9997	0.1	0.5
Fencamfamin	0.484	0.450	0.2–5.0	0.9991	0.9994	0.2	0.5
Fenfluramine	0.259	0.218	0.05–5.0	0.9996	0.9994	0.05	1.0
Fentanyl	1.155	1.061	0.05–3.0	0.9992	0.9945	0.05	0.5
Flecainide[d]	0.829	0.782	0.2–5.0	0.9971	0.9955	0.2	3.0
Fluconazole	0.736	0.791	0.1–3.0	0.9993	0.9973	0.1	0.5
Flumazenil	1.057	1.059	0.05–50	0.9999	1.0000	0.05	5.0
Fluoxetine	0.577	0.526	0.2–5.0	0.9932	0.9932	0.2	3.0
Fluvoxamine	0.589	0.529	0.2–5.0	0.9817	0.9706	1.0	2.0
Haloperidol	1.265	1.235	0.1–3.0	0.9985	0.9632	0.1	0.5
Hydrocone	0.994	1.011	0.1–3.0	0.9995	0.9999	0.1	0.5
Hydroxychloroquine	1.237	1.176	0.5–30	0.9873	0.9848	1.0	5.0
Hydroxyzine	1.221	1.177	0.1–3.0	0.9976	0.9889	0.2	0.5
Imipramine	0.821	0.823	0.05–3.0	0.9997	0.9995	0.05	0.5
Ketamine	0.577	0.604	0.1–3.0	0.9990	0.9916	0.1	0.5
Ketobemidone	0.704	0.729	0.2–5.0	0.9903	0.9905	0.2	1.5
Levomepromazine	1.050	0.995	0.1–3.0	0.9998	1.0000	0.1	0.5
Lidocaine	0.590	0.577	0.1–10	0.9982	0.9981	0.1	2.0
Maprotiline	0.908	0.907	0.2–5.0	0.9724	0.9881	0.2	3.0
MDMA	0.406	0.398	0.1–3.0	0.9965	0.9983	0.2	2.0
Meclozine	1.295	1.252	0.05–5.0	0.9995	0.9999	0.05	0.5
Melperone	0.597	0.557	0.05–3.0	0.9938	0.9944	0.05	0.5
Mepivacaine	0.712	0.728	0.2–10	0.9950	0.9961	0.2	5.0
Mesoridazine	1.547	1.779	0.1–3.0	0.9995	0.9994	0.2	2.0
Metamphetamine	0.237	0.225	0.2–10	0.9997	0.9999	0.2	2.0
Methadone	0.762	0.743	0.05–3.0	0.9999	0.9997	0.05	1.0
Methyl phenidate	0.513	0.513	0.05–5.0	0.9999	0.9997	0.05	0.5
Metoclopramide	1.107	1.087	0.05–3.0	0.9931	0.9991	0.05	0.5
Metoprolol[c,d]	0.684	0.666	0.5–5.0	0.8873	0.8991	0.5	3.0
Mexiletine	0.337	0.321	0.5–10	0.9928	0.9932	0.5	3.0
Mianserine	0.807	0.844	0.05–3.0	0.9983	0.9989	0.05	0.5

Table 3. Continued

Milnasipram	0.931	0.862	0.1–3.0	0.9999	0.9827	0.1	0.5
Mirtazapin	0.840	0.885	0.05–3.0	0.9987	0.9988	0.05	0.5
Moclobemide	0.834	0.877	0.1–3.0	0.9997	0.9999	0.1	3.0
Molindone	1.020	1.023	0.1–3.0	0.9988	0.9995	0.1	1.0
Moperone	1.211	1.138	0.1–3.0	0.9993	0.9984	0.1	0.5
Nefazodone	2.045	[f]	0.1–3.0	0.9964		0.5	0.5
Nicotine	0.313	0.309	0.1–3.0	0.9970	0.9970	0.1	0.5
Nomifensine	0.769	0.850	0.2–10	0.9978	0.9978	0.2	1.0
Norcitalopram	0.982	0.952	0.1–1.0	0.9615	0.9371	0.5	0.5
Norclomipramine	0.987	0.958	0.1–3.0	0.9961	0.9971	0.5	0.8
Nordazepam	1.037	1.044	0.1–5	0.9990	0.9992	0.1	1.5
Nordextropropoxyphene amide	1.065	1.020	0.1–3.0	0.9971	0.9970	0.2	2.0
Nordoxepin	0.837	0.870	0.1–3.0	0.9873	0.9871	0.2	0.5
Norlevomepromazine	1.069	1.025				Qualitative	
Normethadone	0.732	0.723	0.1–3.0	0.9991	0.9991	0.1	1.0
Normianserine	0.841	0.900	0.1–3.0	0.9954	0.9970	0.1	0.5
Norpromazine	0.922	0.944				Qualitative	
Nortramadol	0.647	0.662	0.1–3.0	0.9953	0.9970	0.1	2.0
Nortrimipramine	0.840	0.850	0.1–3.0	0.9980	0.9978	0.2	0.5
Nortriptyline	0.813	0.834	0.1–3.0	0.9978	0.9972	0.1	2.0
Norverapamil[c]	1.421	1.541	0.1–3.0	0.8673	0.8513	1.0	1.0
Noscapine	1.381	1.569	0.1–3.0	0.9990	0.9959	0.2	0.5
Olanzapine	1.159	1.140	0.05–3.0	0.9992	0.9989	0.05	0.5
Orphenadrine	0.626	0.612	0.1–3.0	0.9939	0.9952	0.1	0.5
Oxycone	1.047	1.046	0.1–3.0	0.9989	0.9954	0.1	0.5
Pentazocine	0.859	0.849	0.2–5.0	0.9992	0.9997	0.1	0.5
Pentoxyverine	0.838	0.800	0.1–3.0	0.9982	0.9996	0.1	0.5
Pethidine	0.520	0.500	0.1–3.0	0.9994	0.9995	0.1	2.0
Phenazone	0.582	0.664	0.5–50	0.9897	0.9892	0.5	15
Phencyclidine	0.599	0.565	0.05–3.0	0.9981	0.9993	0.05	0.5
Pheniramine	0.554	0.551	0.05–3.0	0.9968	0.9975	0.05	0.5
Phentermine	0.229	0.219	0.05–5.0	0.9990	0.9991	0.05	3.0
Phenytoin	0.916	0.982	5.0–100	0.9992	0.9848	5.0	15
Pholcodine	1.380	1.532	0.1–3.0	0.9999	0.9943	0.5	0.5
Prilocaine	0.567	0.559	0.1–10	0.9991	0.9991	0.1	5.0
Procainamide	0.830	0.893	0.2–30	0.9973	0.7487	5.0	15
Promazine	0.900	0.909	0.1–3.0	0.9998	0.9982	0.1	2.0
Promethazine	0.862	0.879	0.1–3.0	0.9996	0.9997	0.1	0.5
Propranolol[c,d]	0.768	0.775	0.5–5.0	0.9116	0.9150	0.5	2.0
Quetiapine	1.425	1.604	0.1–3.0	0.9990	0.9910	0.2	0.5
Quinine	1.201	1.187	0.2–10	0.9983	0.9986	0.2	2.5
Reboxetine	0.913	0.942	0.1–3.0	0.9837	0.9846	0.5	0.5
Ropivacaine	0.802	0.793	0.1–3.0	0.9999	0.9999	0.1	0.5
Selegiline	0.353	0.335	0.1–3.0	0.9983	0.9975	0.1	0.5
Sertraline	0.929	0.918	0.1–3.0	0.9988	0.9961	0.1	2.0
Strychnine	1.376	1.593	0.1–3.0	0.9999	1.0000	0.1	1.0
Temazepam[d]	1.229	1.340	0.2–5.0	0.9973	0.9983	0.2	2.0
Thioridazine	1.357	1.438	0.1–3.0	0.9993	0.9994	0.1	2.0
Thioridazine, 5-sulfoxide	1.723	[f]	0.1–3.0	0.9917		0.2	2.0
Tizanidine	1.011	1.081	0.5–5.0	0.9685	0.9727	0.5	2.0
Tramadol	0.630	0.620	0.1–3.0	0.9973	0.9974	0.1	2.0
Tramadol, *O*-desmethyl	0.675	0.688	0.1–3.0	0.9974	0.9958	0.2	2.0
Trazodone	1.501	1.732	0.2–5.0	0.9981	0.9967	0.2	2.0
Trimeprazine	0.880	0.878	0.1–3.0	0.9995	0.9995	0.1	0.5
Trimetoprim	1.082	1.096	1.0–50	0.9972	0.9978	1.0	10
Trimipramine	0.817	0.803	0.1–3.0	0.9999	0.9998	0.1	0.5
Venlafaxine	0.719	0.712	0.1–3.0	0.9980	0.9983	0.1	0.5
Verapamil	1.381	1.445	0.1–3.0	0.9998	0.9999	0.1	1.0
Zaleplon	1.297	1.416	0.05–2.5	0.9877	0.9857	0.1	0.5
Zolpidem	1.202	1.228	0.1–3.0	0.9982	0.9982	0.1	0.5
Zopiclone[c,d]	1.343	1.555	0.1–3.0	0.9976	0.9976	0.02	1.5

[a] Range studied is based on therapeutic and toxic blood concentrations.
[b] Criteria for LOQ: 20% precision and 15% accuracy in the quantitative result of four parallel samples using single point calibration.
[c] Quantitation preferably by another dedicated method.
[d] Several peaks are produced; the main peak is indicated in the table.
[e] Internal standard.
[f] Not analysed on this column.

prescription drugs relevant in forensic toxicology that can be analysed without prior derivatisation by GC even at the therapeutic concentration level. The method has been used in the routine toxicological screening of medical examiner's cases for a 1-year period and it has been accredited by the Finnish Centre for Metrology and Accreditation (FINAS). The maintenance has included shortening the pre-columns weekly by 50 cm and quantitative calibration in 1-month intervals. Fig. 1 shows a typical pair of chromatograms obtained at casework, indicating poisoning with the antipsychotic drug levomepromazine (methotrimeprazine) while Fig. 2 shows chromatograms of blank autopsy blood.

During the last decade, only a few methods based solely on GC have been suggested for comprehensive drug screening purposes. Instead, reports applying GC–MS have been more common, the emphasis in these methods being usually on spectral identification. However, in the present application, GC was considered more feasible than GC–MS for the following reasons: All basic drugs contain nitrogen and consequently they are amenable to sensitive nitrogen selective detection. In addition, dual column GC allows the use of three independent identification parameters: the retention parameter on the first column, the retention parameter on the second column, and comparison of the quantitative response factors (the concentrations found) on the two columns. GC–MS in the full scan mode is generally not sensitive enough to detect basic drugs at therapeutic concentrations in the blood. In the selected ion monitoring mode, GC–MS suffers from unspecific fragmentation common to many basic drugs. There are also both hardware and software limitations in handling large libraries of selected ions.

Recently, the status of retention time-based identification has been restored by advances in GC tem-

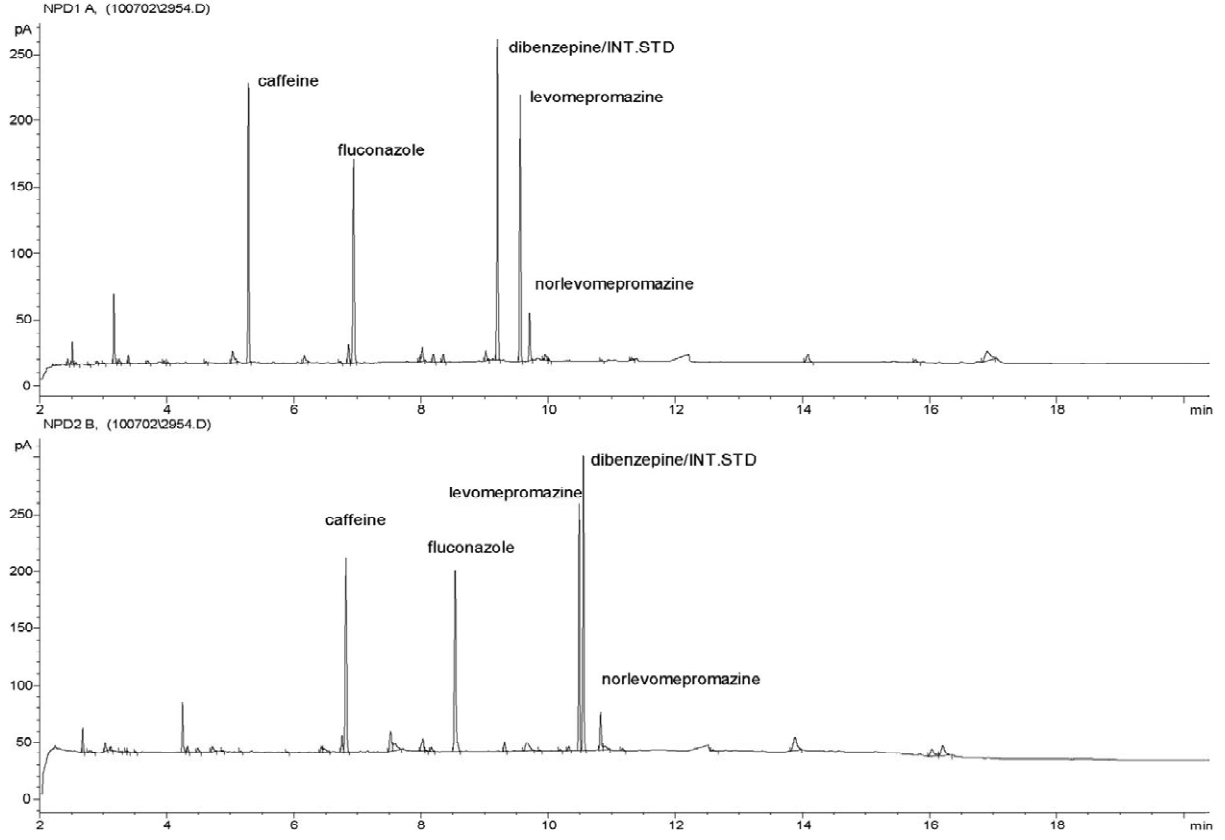

Fig. 1. Analysis of basic drugs in autopsy blood from a poisoning case on HP-5 (above) and DB-17 (below). Findings: caffeine 3.0 mg/l, fluconazole 2.3 mg/l, levomepromazine (methotrimeprazine) 1.2 mg/l, norlevomepromazine positive.

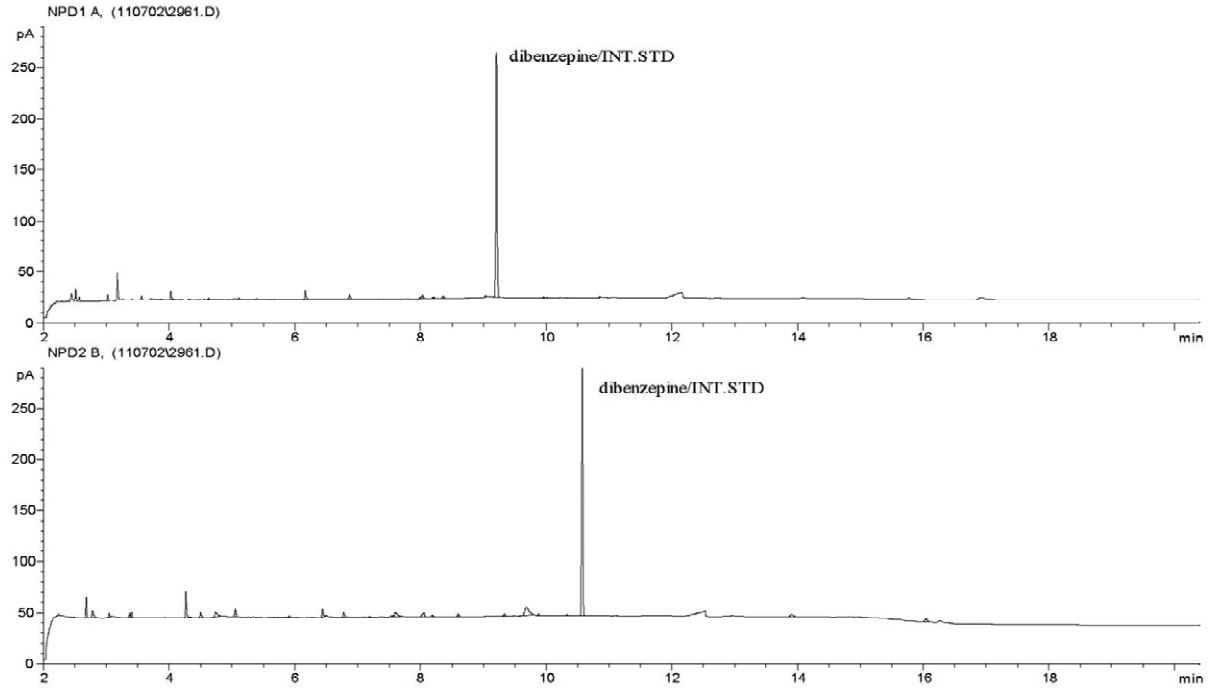

Fig. 2. Analysis of blank autopsy blood containing the internal standard dibenzepin (1.0 mg/l).

perature and pneumatics control, leading to approaches like fast chromatography and method translation [11,12]. The present study adds to this development by showing the significance of RTL in improving the long-term precision within a method on a single instrument, thus making identification feasible without special RI techniques. In general, GC-based techniques are superior to LC and LC–MS in terms of chromatographic separation power, analysis costs, and ease of maintenance. However, for systematic toxicological analysis, several complementary techniques are inevitably needed.

4. Conclusions

This study shows that use of the RTL function significantly improves the precision of identification, not only between systems or laboratories as has already been known, but also within an individual GC instrument and column. In this type of application the constant flow mode can be used. The high precision obtained with RTL allows compound identification based on the relative retention time, even for the absolute retention time, instead of the more complicated retention index system. These features will strengthen the position of GC-based techniques in the area of comprehensive drug screening.

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