Journal of Pharmaceutical & Biomedical Analysis
Vol. 8, Nos 8–12, pp. 655–661, 1990
Printed in Great Britain

0731–7085/90 $3.00 + 0.00

Indirect continuous automatic determination of pharmaceuticals by atomic absorption spectroscopy*

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Abstract: The implementation of continuous separation techniques such as precipitation, liquid–liquid and solid–liquid extraction in FIA manifolds coupled on-line with an atomic absorption spectrometer for the determination of active components (sulphonamides, local anaesthetics, amphetamines, benzodiazepines, chloramphenicol and methadone) in pharmaceuticals and biological fluids is systematically described. The basic features of the analytical methodologies described (sensitivity, selectivity, precision and rapidity) are also discussed and critically compared.

Keywords: *Sulphonamides; local anaesthetics; amphetamines; benzodiazepines; chloramphenicol; methadone; continuous separation techniques; atomic absorption spectroscopy.*

Introduction

A number of analytical methods described in current Pharmacopoeias rely on the use of metal ions as reagents for the determination of a variety of active components in pharmaceutical preparations. Most of such methods are time-consuming and occasionally involve outdated analytical techniques. Nevertheless, the use of indirect atomic absorption methods for determination of organic analytes is well recognized as a useful alternative in this context [1]. These methods rely on the development of an ion-pair or complex formation, a redox or precipitation reaction between the organic-pharmaceutical species and the cationic reagent. The most serious drawbacks are the high degree of human participation required, the high cost involved and the irreproducibility of the results obtained.

Consequently, the automation of these analytical methodologies is very interesting and promising as it should make them competitive with corresponding manual alternatives for the determination of the same analytes in terms of sensitivity, selectivity, precision, rapidity and economy. Flow injection analysis (FIA) is by now an extensively used approach to automate non-chromatographic continuous separation techniques (e.g. solid–liquid, liquid–liquid extraction, precipitation) [2–4]. Thus, the on-line coupling of an FIA manifold with an atomic absorption spectrometer (see Fig. 1) allows the indirect AAS determination of active components in pharmaceutical preparations.

This paper describes the use of precipitation, ion-pair formation (with liquid–liquid extraction) and redox (using solid redox agents) reactions used in these hybrid configurations

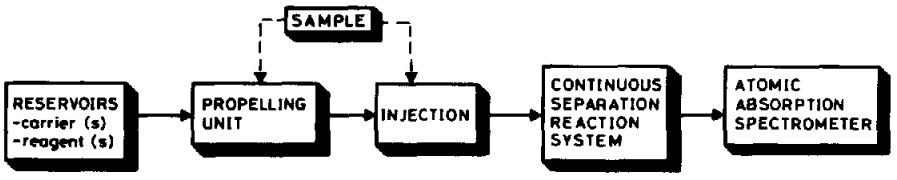

Figure 1
Schematic diagram of an FIA manifold including a continuous separation–reaction system coupled on-line with an atomic absorption spectrometer for the indirect determination of pharmaceuticals.

*Presented at the "Second International Symposium on Pharmaceutical and Biomedical Analysis", April 1990, York, UK.

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and their application to the determination of a variety of organic compounds in pharmaceuticals.

Experimental

Apparatus

An atomic absorption spectrometer (Perkin–Elmer 380) equipped with suitable hollow-cathode lamps. Two peristaltic pump (Gilson Minipuls-2) furnished with poly(vinyl chloride) tubes. An injector (Tecator L 100-1) and a selecting valve (Rheodyne 5041) were used. A Scientific System 05-105 column with a removable screen-type stainless-steel filter (pore size, 0.5 μm; chamber inner volume, 580 μl; and filtration area, 3 cm^2) which was originally designed as a cleaning device for HPLC, was employed for filtration purposes. An A-10 T solvent segmenter and a phase separator with Fluoropore membrane (1.0 μm pore size, FALP 04700, Millipore) was employed for continuous extraction. The reduction columns were conditioned by packing a glass capillary (8.5 cm long, 1.8 mm bore) with cadmium or zinc granules of medium size.

Reagents

Sodium sulphonamides and local anaesthetics were obtained as certified pharmaceutical samples (≥99.5% pure) and dissolved (stock solutions 1.000 g l^{-1}) in distilled water. Solutions of amphetamines and bromazepam (Sigma) were prepared (stock solutions 1.000 g l^{-1}) in distilled water and methanol, respectively; methadone hydrochloride (Sigma) (stock solution 1.000 g l^{-1}) in distilled water; chloramphenicol (Sigma) by dissolving 1 g of the dried powder in the minimum amount of ethanol and diluting to 1 l with 1:1 (v/v) ethanol–water. Chlordiazepoxide hydrochloride (Sigma) was dissolved (stock solution 1.000 g l^{-1}) in ethanol. Others reagents employed were of analytical reagent grade.

Continuous Precipitation

The usual operation of a continuous precipitation system involves inserting the sample containing the analyte and a precipitating reagent solution (usually an inorganic cation). On mixing, the two solutions yield a precipitate which is retained on a suitable filter. The filtrate, then flows to the detector [5]. The simplest possible configuration is the reversed

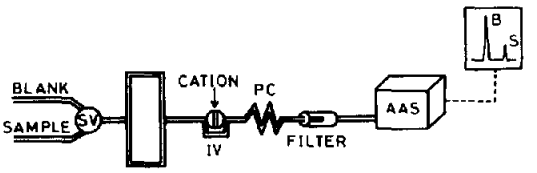

Figure 2
Manifold for continuous precipitation of sulphonamides and local anaesthetics, coupled on-line to an atomic absorption spectrometer. IV, injection valve; SV, selecting valve; PC, precipitation coil.

flow-injection manifold shown in Fig. 2, which includes a selecting valve to make blank measurements. First, the reagent cation is injected into a water stream and a high FIA peak is obtained. The selecting valve is then switched and the sample is continuously pumped into the system; another identical injection of the reagent causes the analyte to form a precipitate which is retained on the filter. The FIA peak obtained decreases with increasing analyte concentration in the sample. The system occasionally includes a water stream prior to the nebulizer for dilution of the reagent cation if its concentration falls outside the linear range of the instrument.

General features

The efficiency of a continuous precipitation system is directly influenced by the flow rates, injected volume and geometric features of the precipitation coil and by the type of filter used. The absorbance usually increases with increase in the amount of reagent cation injected into the water (blank) or the sample. Therefore, both the blank and the sample signal are dependent on the plug width (as a result of the increased reaction zone and longer filtrate plug), but the difference between them remains constant for 100–200 μl of injected cation solutions. The coil length significantly influences the precipitation efficiency, thus, with short coils (usually <100 cm), the reaction is incomplete because the contact time between the reagent cation and the analyte is too short. The flow rate of the sample-carrier is dictated by the nebulizer, and should be higher than 2 ml min^{-1}. Flow rates between 2.5–3.5 ml min^{-1} are the most frequently used in these continuous precipitation systems.

The filters should ideally be cleaned with the same reagent used for the precipitate dissolution in ultrasonic batches for 1–3 min at intervals depending on the analyte concen-

tration in the samples (usually after 150–200 samples).

Determination of sulphonamides and local anaesthetics in pharmaceutical preparations

By using the flow manifold depicted in Fig. 2, sulphonamides in pharmaceutical preparations were determined [6] by precipitation with copper (copper method) or silver (silver method) ions at pH 6–7, over the range 1.5–35 μg ml^{-1}, with a relative standard deviation (RSD) between 1.5–3%, at a sampling frequency of 100–150 h^{-1}. Different substances potentially found in pharmaceutical samples as excipients and diluents (glucose, lactose, sucrose, ethylene glycol, glycerol, vanillin, talc powder and starch) did not interfere. The copper method was more selective (chloride and phosphate did not interfere) than the silver method and can be applied to the determination of sulphonamides in urine.

By using a similar configuration to that employed for the determination of sulphonamides local anaesthetics (lidocaine, procaine and tetracaine) were assayed in pharmaceuticals [7]. A preliminary study of the solubility of different metal ions with these drugs in aqueous solutions at various pH values indicated that only cobalt ions formed sufficiently insoluble precipitates with the above-mentioned local anaesthetics in weakly basic media. The optimization of all the variables involved allowed determination over the range 2.0–35 μg ml^{-1} with high precision (RSD 0.5%, $n = 11$). Ions commonly found in biological fluids (Cl^-, PO_4^{3-}, SO_4^{2-}, NO_3^-, Ca^{2+}, Mg^{2+}, Na^+, K^+, Mn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}) up to 1 mg ml^{-1} for 10 μg ml^{-1} lidocaine hydrochloride had no effect in 100-fold excesses. Some substances found in pharmaceuticals such as monophosphotiamine, cyanocobalamin, pyridoxine, vitamin C, methanosulphonate sodium noramidopyrine, di-isopropylammonium dichloroethane, metampiciline, cloxacillin, bromexine, cocarboxylase, pyridoxal, penicillin G, phenazone, boric acid, nafazoline and adrenaline did not interfere. This is demonstrated by the acceptable recoveries of the anaesthetics in these matrices (Table 1).

The two methods for the indirect determination of sulphonamides (precipitation with copper) and local anaesthetics (precipitation with cobalt) were applied to different compounds. The results obtained are summarized in Table 1.

Continuous Liquid–liquid Extraction

Two types of continuous extraction system have so far been coupled to an atomic spectrometer detector. In one, devised by Nord and Karlberg [8] and featuring no sample injection, the sample is continuously introduced into the extraction system. The organic phase stream emerging from the separator at a flow rate below 1 ml min^{-1} is linked to an injector loop, the content of which flows into a water stream leading to the AAS instrument at a flow rate of 4–5 ml min^{-1}. This ensures the best possible performance from the spectrometer. In the other alternative, with sample injection, it is always the sample which is injected into the system. In this case, the difference between the nebulizer aspiration rate (4–6 ml min^{-1}) and the flow rate of organic solvent across the membrane (1–2 ml min^{-1}) is compensated for by an additional stream of pure solvent or air inserted via a T-piece. The former configuration provides higher sensitivity than the latter as it involves no dilution of the organic extract prior to the measurement.

Table 1
Determination of sulphonamides and local anaesthetics in pharmaceutical preparations

Pharmaceutical	Nominal content	Average recovery (%)*
Bronquimucil (sulphamethoxazole)	0.4 g/capsule	97.9 ± 1.5
Sulphintestin (formilsulphatiazole)	0.4 g/tablet	101.2 ± 1.3
Micturol (sulphamethizole)	0.125 g/tablet	101.6 ± 0.9
Bio-Hubber (sulphadiazine)	5.0 mg ml^{-1}	99.8 ± 0.9
Oculos (sulphacetamide)	0.1 g ml^{-1}	99.6 ± 0.5
Menalgil Plus (lidocaine)	3.3 mg ml^{-1}	99.8 ± 0.6
Bronco Pensusan (lidocaine)	8.0 mg ml^{-1}	100.3 ± 0.8
Penibiot (lidocaine)	2.0 mg ml^{-1}	99.7 ± 1.0
Dentol Tópico (procaine)	10.0 mg ml^{-1}	100.0 ± 0.4
Otosedol (procaine)	12.6 mg ml^{-1}	100.2 ± 0.7

*$n = 5$.

The analytical potential of indirect determinations based on the formation of ion-pairs and the use of FIA–AAS in conjunction with liquid–liquid extraction has been clearly shown in recent papers [9–11]. Therefore, a continuous extraction system without sample injection, coupled to an atomic absorption spectrometer was used to determine bromazepam and amphetamines in pharmaceuticals and biological fluids.

General features

The indirect determination of bromazepam is based on its basic dipyridyl type bond structure, which has excellent complexing capabilities with divalents ions such as copper, cobalt, zinc and iron(II). These complexes can be readily extracted into methyl isobutyl ketone (MIBK) with perchlorate as counterion and the metals can be measured in the organic extract.

Amphetamine and methylamphetamine (secondary amines) react with carbon disulphide in aqueous ammonia to give dialkyldithiocarbamic acid derivatives. In the presence of nickel, copper or zinc, the corresponding complex is formed and subsequently extracted into MIBK.

The automation of the batch procedures for the indirect determination of these drugs by AAS associated with a flow injection technique is influenced by factors such as flow injection variables (e.g. flow rates of aqueous and organic phases, sample volume injected, long of the extraction coil, etc.) and chemical variables. With the manifold depicted in Fig. 3, the sample (amphetamine or bromazepam) is continuously pumped into the system and mixed with the carrier S_2C and 75 μg ml^{-1} of metal ions (Cu^{2+}, Ni^{2+} or Zn^{2+}) in ammonia or 1 M perchlorate and 25 μg ml^{-1} of copper in acetic acid/acetate buffer for amphetamines or bromazepam, respectively. A stream of MIBK and the mixed sample-carrier solution stream are segmented by the solvent segmentor. The extraction process takes place in the extraction coil and a fraction of the extract is separated through a membrane phase separator. Amphetamines and bromazepam were determined by injecting 100–150 μl of organic extract through injection valve IV into a water stream aspirated by the nebulizer.

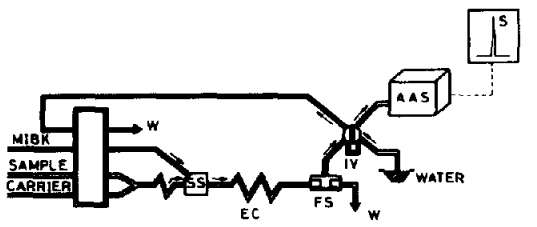

Figure 3
Continuous liquid–liquid extraction system for the indirect determination of amphetamines and bromazepam. SS, solvent segmentor; EC, extraction coil; FS, phase separator; W, waste.

The efficiency of an extractive procedure is particularly influenced by the injected volume, geometric features of the extraction coil and flow rates. The extracted sample volume injected into the water line had a significant effect on the absorbance.

The signal increased with volumes up to 70–100 μl, above which it remained constant because of the constant atomization efficiency achieved.

The optimum extraction coil length is proportional to the contact time between phases required for the extraction of the ion-pair or complex into the organic phase. As continuous extraction systems do not require chemical equilibrium to be reached, their contact times need only be a fraction of those demanded by manual procedures. An extraction coil length of 300–500 cm was required for the extraction (80–90%) of amphetamines and bromazepam.

The number of possible flow rate combinations in the extraction system was quite large since several of the flows could be varied individually. The absorbance generally increased as the flow ratio of sample to organic phase increased due to increase in the preconcentration ratio. A sample flow rate of 3.4 ml min^{-1} and an organic phase flow rate of 0.5 ml min^{-1} was chosen for the determination of amphetamines; taking into account the mutual influence of three factors: reproducibility (inversely proportional to the flow rates), concentration ratio and sampling frequency. In the determination of bromazepam, sample to organic phase experiments carried out at flow ratios between 1 and 7.5 at different concentrations of bromazepam revealed that, surprisingly, the concentration of extracted ion-pair did not increase with increasing flow-rate ratio, but remained constant throughout.

Determination of amphetamines and bromazepam in pharmaceuticals and biological fluids

With the manifold depicted in Fig. 3,

amphetamine and methylamphetamine were determined over the range 0.01–1.0 μg ml^{-1} (R. Montero, unpublished results). A number of compounds of the diazepines series, pentobarbital, morphine, cocaine and methadone did not interfere up to a concentration of interferent of 100 μg ml^{-1} for 0.5 μg ml^{-1} methamphetamine.

The determination of bromazepam by AAS involved reaction with copper to form an MIBK extractable ion-pair with perchlorate (R. Montero, unpublished results). The absorbance of the copper in the extract was linearly related to bromazepam concentrations between 0.1–4.0 μg ml^{-1}. The procedure yielded acceptable results and was subject to no interference from compounds such as chlordiazepoxide, nitrazepam, diazepam, oxazepam, flurazepam, codeine, pentobarbital or methamphetamine at concentrations 1000-fold higher than that of bromazepam.

Both procedures were applied to the determination of bromazepam in plasma and amphetamine in urine; the recoveries ranged from 96.4 to 103.2% (n = 5). Also, the methods were used for the determination of these drugs in some pharmaceutical preparations, Centramina (amphetamine sulphate, 10 mg/tablet) and Lexatin (1.5, 3 or 6 mg/capsule of bromazepam), with excellent results.

The method compares favourably with its batch counterpart and has several advantages (Table 2) which can be classified into two groups:

(1) Those inherent in the FIA technique: small sample volume and low reagent consumption, higher sampling frequency and low cost per determination.
(2) Specific: wide pH range; no blank required; higher sensitivity, selectivity and precision (coefficient of variation).

Solid-phase Redox Columns

Oxidizing and reducing substances can sometimes be determined with either one or two reagents (one of which is a metal ion) that can react with the substance in question. In general, these methods are not very selective because complete absence of other oxidizing or reducing agents is required. Some organic compounds have also been determined indirectly by redox reactions. These procedures require using a metal to reduce an organic species, the amount of oxidized metal being measured by AAS. Alternatively, a metal ion can be reduced to its elemental form and the precipitated metal can be dissolved into nitric acid and determined by AAS, or the change in the concentration of metal ion be measured, also by AAS. In general, existing procedures have been applied to functional groups such as aldehydes, alcohols, nitrocompounds, sugars and folic acid [1]. Hassan and Eldesouki [12] determined chloramphenicol compounds in pharmaceuticals by reducing the NO_2 group with cadmium metal and measuring the released cadmium ion by AAS. However, the reduction process involves extensive manipulation, a high consumption of cadmium metal (50–100 mg per sample) and boiling for 15–20 min under a carbon dioxide atmosphere. The nitro group in organic compounds has also been determined using AAS by reducing the analyte to its hydroxylamine derivative with zinc powder [13]. The method involves several steps, is rather tedious and time-consuming and subject to severe interferences.

The automation of redox systems was approached by exploiting the advantages of flow injection analysis, particularly its simplicity and versatility [14]. Different redox columns were coupled on-line with a conventional atomic absorption instrument for the selective determination of organic compounds. The reduction columns were prepared by packing a glass capillary with cadmium or zinc granules. Columns of copper-coated cadmium or amalgamated zinc were also prepared. The most simple configuration is shown in Fig. 4.

Table 2
Determination of amphetamine and bromazepam by extraction into methyl isobutyl ketone

	Amphetamine		Bromazepam	
	FIA method	Batch method	FIA method	Batch method
Sample pH	4.5–8.0	0.1 N NH_3	3.7–9.8	4.0–5.4
Aqueous/organic phase ratio	7	0.16	2	1.3
Sensitivity (ml μg^{-1})	1.18	0.017	0.05	0.031
Coefficient of variation (%)	1.7	2.5	1.7	2.8
Sampling frequency (h^{-1})	40	5	50	10

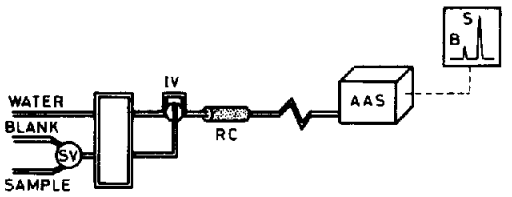

Figure 4
Redox system for the reduction of organic compounds. RC, redox column.

The sample, in an acid medium, was injected into a water-carrier. Firstly, a water blank at the same pH as the sample was injected to obtain a smaller peak due to the dissolution of the metal from the column (cadmium or zinc) by the acidic medium. Secondly, the sample was injected to obtain a higher peak due to the redox reaction. The difference between the two peaks was proportional to the injected drug concentration.

General features

The system depicted in Fig. 4 was used to assay various organic compounds bearing functions potentially reducible with these redox systems, namely: sulphone (dapsone, 4-4′-sulphonil-bisbenzenamine), which could be reduced to diphenyl sulphoxide or diphenyl sulphide; chlordiazepoxide (librium), the only 1,4-benzodiazepine which contained an easily reducible *N*-oxide group; methadone (structurally a γ-keto-tertiary amine) reducible in its keto group; and chloramphenicol, with a nitro group which can be readily reduced to its hydroxylamine derivative by cadmium or zinc metals, as widely investigated through manual procedures [12]. The continuous assays showed all these compounds except the dapsones to be readily reduced.

The redox reactions involved were influenced by the pH of the medium, they were feasible in acid media only. However, below pH 3.5, the blank signal fell outside the linear range of the instrument owing to the dissolution of the metal (cadmium or zinc) in the acid medium. The injection of water samples into an acid carrier was inadvisable as it shortened the life of the columns; thus, we opted for injecting pre-acidified samples into a distilled water-carrier. The temperature for these reactions, studied in the range 10–80°C, did not affect the reaction yield. The column length was also not influential as the drugs were reduced instantaneously. Since the redox reaction took place inside a narrow-bore column, the automatic procedure was efficiently shielded from the transfer of substances from the atmosphere to the sample and vice versa; no CO_2 atmosphere was thus required as the continuous system was closed. The columns had a useful life of 1 month under continuous use.

Flow injection variables such as the injected volume and flow rate of the water-carrier did not affect the performance of the continuous redox system.

Determination of different drugs by continuous redox reactions

By using the continuous redox system depicted in Fig. 4, various drugs were determined by reduction with metal cadmium or zinc. The optimum conditions and analytical data are summarized in Table 3.

The method for determination of chlordiazepoxide [14] is selective towards this compound over 1,4-benzodiazepines such as diazepam, oxazepam, medazepam, flurazepam, bromazepam, lorazepam and nitrazepam. These drugs were tolerated at concentrations 100 times higher than that of chlordiazepoxide with the exception of nitrazepam, which was tolerated at concentrations only 5 times higher. This interference arose from the reduction of the nitro group to the corresponding amine.

Table 3
Optimum conditions and analytical data for the determination of various drugs

Parameter	Chlordiazepoxide	Methadone	Chloramphenicol
Sample pH	3.5–5.0	3.3–4.3	3.7–4.2
Injected volume (μl)	200	90	90
Water flow rate (ml min^{-1})	3.5	3.0	3.0
Linear range (μg ml^{-1})	2–25	5–50*	2–30
Detection limit (μg ml^{-1})	1.0	3*	1.0
Coefficient of variation (%)	1.5	1.8	1.4
Sampling frequency (h^{-1})	150	150	150

*ng ml^{-1}.

The method was applied to the determination of chlordiazepoxide in dosage forms, with recoveries between 97.3–102.1% (n = 5).

The sensitivity and detection limit of the method for the determination of methadone surpasses that of other alternative methods for clinical samples [15]. The higher sensitivity arises from the fact that the amine group is protonated in acidic media, which significantly facilitates the reduction of the keto group. Other drugs of social interest such as codeine, papaverine, morphine, heroin, cocaine and 1,4-benzodiazepines were tolerated at concentrations 1000-fold that of methadone. The method can be applied to the determination of methadone in urine samples [15].

The determination of chloramphenicol [16] has clear advantages over its batch counterpart: higher sensitivity (linear range 2–30 μg ml^{-1} for the FIA technique and 2–15 mg ml^{-1} for the batch procedure); higher precision because of the lower sample manipulation involved; higher sampling frequency (the batch method requires boiling for 15–20 min) and low cost per determination. The determination of chloramphenicol and its esters in various pharmaceutical preparations is not interfered with by additives or diluents commonly used in drug formulations. The average recovery for the determination of chloramphenicol in capsules, tablets, syrup and ointments ranged between 97.2–102.8% (n = 5).

Acknowledgements — The authors wish to express their gratitude to the CICYT for financial support received (Project No. PA86-0146).

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[*Received for review 5 April 1990; revised manuscript received 23 April 1990*]