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Trimipramine determination in pharmaceutical preparations with an automated multicommutated reversed-flow system

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Abstract

In this work an automated multicommutated flow methodology was implemented for the spectrophotometric determination of trimipramine in pharmaceutical preparations by oxidation with ammonium monovanadate in acidic medium. The developed procedure exploits a new approach for sample/reagent intermixing by combining binary sampling with flow-reversal. Rather than inserting the sample as a single continuous volume the intercalation of multiple small sample and reagent aliquots, under a time-based control, created multiple reaction interfaces that promoted reaction zone homogenisation even in limited dispersion conditions. Additionally, the reaction interfaces were reversed, increasing mutual zone penetration, which contributed to a faster reaction development while assuring a low dispersion pattern. A linear range of determination was verified for trimipramine concentrations between 1.0 and 18.0 μg ml^{-1} with a relative standard deviation ($n = 10$) lower than 1.69% and a sample throughput of about 26 samples per hour. The results were in agreement with those obtained by the reference procedure with relative deviations lower then 2.37%.

Keywords: Trimipramine; Multicommutated flow system; Reversed flow; Binary sampling; Spectrophotometry

1. Introduction

One of the many features that could be used to characterise or to establish similarities or distinctions among continuous flow techniques is the strategy employed for sample/reagent mixing. While in flow injection analysis (FIA) [1] the sample zone is usually injected into a flowing stream that subsequently merges with converging reagent streams on the way to detection, in sequential injection analysis [2] a sample zone and a reagent zone are sequentially inserted and stacked into a holding channel originating a reaction zone that is subsequently carried out through the reactor into the detector by flow-reversal. In both situations the efficiency of the mixing approach is of primordial importance for convenient reaction development and usually involves a sample/reagent mutual dispersion within an adequately sized reaction coil not only to

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improve the analytical signal but also reproducibility.

Multicommutated flow systems [3] relying on the utilisation of multiple mono-commutating devices (solenoid valves) enable the exploitation of a distinct approach for sample and reagent insertion that consisted on the intercalation of very small sample and reagent aliquots (binary sampling) under a time-based control. This approach produces multiple reaction interfaces for each sample solution, which coalesce during transport to detection. Nevertheless, despite the enhanced sample/reagent mixing achieved a reaction coil is always required to promote additional sample dispersion.

In this work, by combining the mixing potential of binary sampling and the zone penetration attained with flow-reversal [4], fast and improved homogenisation was achieved, even with highly viscous solutions, which permitted to significantly reduce the size of the reaction coil or even its virtual suppression, while assuring high reproducibility, sensitivity and sampling rate and low reagent consumption.

Trimipramine maleate, 5-(3-dimethylamino-2-methylpropyl)-10,11-dihydro-5*H*-dibenz [*b*,*f*] azepine acid maleate, is a tricyclic antidepressant agent with an anxiety-reducing sedative activity that substantiates its efficacy in the treatment of primary insomnia. Due to its pharmacological profile, trimipramine might also be active as an antipsychotic. The therapeutical and pharmacological relevance of trimipramine, in addition to its inherent toxicity has prompted the development of several methods for its determination both in pharmaceutical preparations and biological samples, including liquid chromatography [5], conductimetry [6], chemiluminescence [7,8], spectrophotometry [9] and voltammetry [10,11]. However, most of the already available methods are either time consuming or require relatively expensive equipment. In fact, the reference method of the European Pharmacopoeia [12] involves a non-aqueous potentiometric titration of trimipramine with 0.1 N perchloric acid, in glacial acetic acid. Moreover, the advantageous features associated to the implementation of automated analytical methodologies were scarcely explored since only a flow-injection (FIA) methodology, a technique that do not exhibit neither the versatility nor the degree of automation of multicommutation, was proposed for trimipramine determination.

Preliminary experiments aimed at exploiting the reactivity of the chemically active nitrogen atoms in the dibenzoazepine structure of trimipramine revealed that this compound reacts with strong oxidants in acidic medium yielding blue-coloured products with a maximum of absorbance at 620 nm. This reaction was used to develop a multicommutated flow system with enhanced analytical capabilities arising from the binary sampling flow-reversal approach, which was applied to the implementation of a fast, reliable and low reagent consumption automated method for the spectrophotometric determination of trimipramine in pharmaceutical preparations.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and doubly deionised water was used.

A 400 mg l^{-1} solution of trimipramine was daily prepared by dissolving 55.78 mg of trimipramine maleate in 100 ml of 4.0 mol l^{-1} sulphuric acid. This solution was kept in the refrigerator. Working standards were prepared by appropriately diluting the above solution with sulphuric acid 4.0 mol l^{-1}.

An 8.9×10^{-3} mol l^{-1} ammonium monovanadate solution was prepared by dissolving 104.4 mg in 4.0 mol l^{-1} sulphuric acid, and completing the volume to 100 ml with the same acid solution.

Sample solutions were prepared from commercially available pharmaceutical preparations (Surmontil tablets dosed 25 and 100 mg) by dissolving the appropriate amounts of powdered tablets with sulphuric acid 4.0 mol l^{-1}. Sample solutions analysed by the developed procedure were not subject to any sample pre-treatment.

2.2. Equipment

A 6100 Jenway spectrophotometer (Jenway, UK) equipped with an 18 μl inner volume flow-cell was used for absorbance measurements at 620 nm.

The flow manifold included four 161 T031 (NResearch, USA) solenoid valves: two 3-way (two inlets and one outlet) and two 2-way (one inlet and one outlet). Flow lines and holding coil were made from 0.8 mm i.d. PTFE tubing. Home-made end-fittings, connectors and confluence points were also used. A Crison Micro BU 2030 automatic burette (syringe-pump) equipped with a 5.0 ml syringe and controlled by a microcomputer through serial protocol (RS-232C) was used to aspirate and propel (bi-directional flow) the solutions through the system. A power drive based on a ULN 2003 integrated circuit was used to operate the solenoid valves [13]. Analytical system control was achieved by means of a PC-LABCard model PCL-818L interface card from Advantech. A single Pentium 200 MHz microcomputer and a unique data program performed the overall analytical system control that included the synchronized operation of the syringe-pump and solenoid valves and data acquisition and processing. The software was developed in BASIC and enabled the multiple controls of the solenoid valves and automatic syringe-pump operation, through a single user order.

2.3. Flow manifold

The developed flow system (Fig. 1) comprised four solenoid valves: two 2-way-normally closed (V_1 and V_2) and two 3-way (V_3 and V_4). The syringe-pump had also a valve (V_p). In the early experiments, the flow manifold comprised two reaction coils (L_1 and L_2), but during system optimisation, as it will be later discussed, it was decided to remove L_2.

The syringe-pump (P) was always filled with sulphuric acid 4.0 mol l^{-1}, used as carrier solution (C) and to establish baseline, and never in contact with reagent (R) or sample solutions (S).

Each individual analytical cycle started with the introduction of the sample solution. Sample was

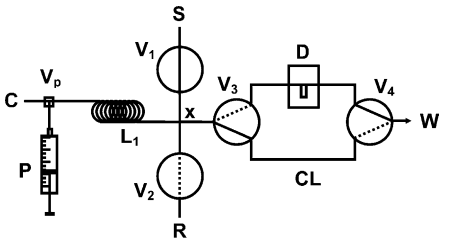

Fig. 1. Diagram of the flow manifold for trimipramine maleate determination. V_1 and V_2—2-way solenoid valves; V_3 and V_4—3-way solenoid valves: solid lines inside the valve symbols correspond to position 1 and dashed lines to position 2; C—carrier solution (sulphuric acid); S—sample; R—reagent (ammonium monovanadate); P—syringe-pump. V_p—syringe-pump valve; L_1—reactor (2 m long); x—confluence point; D—detector; CL—cleanup channel; W—waste.

inserted by means of the alternating actuation of valves V_1 and V_2, at a pre-set timing and sequence, in order to enable the intercalation, by aspiration, of very small aliquots of sample and reagent that filled the reactor L_1. This way the sample was not introduced as a unique volume but as a tandem stream of small sample and reagent segments. The number and timing of each intercalation cycle defined the final sampling time (t_s), which, in addition to the flow rate, determined the whole inserted sample volume. During the insertion cycle valves V_3 and V_4 were always in the same position (position 1) to provide a closed-end and to guarantee that the negative aspirating pressure was only applied at the sample and reagent tubing.

After sample insertion valves V_1 and V_2 were closed, V_3 was actuated to position 2 and the syringe-pump mode was reverted from aspiration to propelling, with the concomitant flow-reversal that re-directed the sample zone within L_1 towards detection (D). The cleansing and sample replacement was performed through channel CL by actuating V_3 to position 1 and V_4 to position 2.

Sampling rate was improved using a variable programmed flow of the syringe-pump. During the filling up phase and during sample replacement and cleanup the movement of the piston was at the maximum speed (highest attainable flow rate) but during sample aspiration and detection the speed was the one determined by system optimisation.

2.4. Reference procedure

The reference method, as recommended by the European Pharmacopoeia [12], involved the dissolution of defined amounts of trimipramine maleate in glacial acetic acid followed by non-aqueous potentiometric titration with 0.1 N perchloric acid.

3. Results and discussion

Preliminary experiments revealed that trimipramine maleate reacts with strong oxidants, in acidic medium, yielding intensively coloured species with an absorbance maximum at 620 nm. The blue oxidation products exhibited different stability and reaction kinetics depending not only on the oxidising agent but also on the type and acid concentration used. Batch experiments showed that ammonium monovanadate was the most appropriate oxidant because it originated stable oxidation products with high reaction's rate, being as well a long-standing easily obtainable reagent. At the same time it was also verified that the reaction was enhanced in acid medium, rather in sulphuric acid than in hydrochloric or nitric acids, and that it depended on the acid concentration.

For the implementation of this reaction in a multicommutated flow system it was designed a flow manifold comprising, initially, two distinct reactors. One of the reactors (L_1), placed before the confluence point, had the primary function of holding the aspirated sample and reagent slugs and was enough lengthy as to prevent the sampling zone to reach the syringe-pump. Additionally, as it was later confirmed, it had a major contribution to the sample zone homogenisation. The second reactor (L_2) was placed just before detection and was aimed at extending the sample/reagent intermixing as well as at incrementing reaction development by increasing reaction time. The length of these reactors was 2.0 and 1.0 m, respectively. Flow rate was set at 1.5 ml min^{-1} and the sampling time (t_s) was 4 s, which established a sampling volume of about 100 μl. In the early experiments, the sample was introduced as a single volume stacked between two identical volumes (100 μl) of reagent solution (insertion time of 4 s each) originating two reaction interfaces. A flow-reversal was used in order to increase sample/reagent mixing at the two sample boundaries. With this manifold configuration the influence of sulphuric acid was assessed at concentrations ranging from 1.0 to 6.0 mol l^{-1} by inserting a 20.0 μg ml^{-1} trimipramine solution. The sulphuric acid solutions were used not only as carrier but also to prepare the trimipramine standards and the reagent solution, which was a 6.0×10^{-4} mol l^{-1} ammonium monovanadate solution, preventing this way the occurrence of the Schlieren effect [14]. The obtained results showed that at concentrations between 1.0 and 3.0 mol l^{-1} no signal was obtained. From 3.0 to 5.0 mol l^{-1} the signal showed a pronounced increase while between 5.0 and 6.0 mol l^{-1} it approached stabilisation. Considering that the analytical signal increment between 4.0 and 5.0 mol l^{-1} was not good enough as to compensate the corresponding viscosity increase, which represented an additional hindrance for reaction zone homogenisation, a 4.0 mol l^{-1} sulphuric acid concentration was selected for posterior experiments.

The influence of ammonium monovanadate was evaluated by using the same manifold configuration at a concentration range between 1.0×10^{-4} and 4.0×10^{-3} mol l^{-1}. By inserting a 50.0 μg ml^{-1} trimipramine solution (at a t_s of 4 s corresponding to 100 μl) and by using 4.0 mol l^{-1} sulphuric acid as carrier it was observed that the analytical signal markedly increased with the ammonium monovanadate concentration from 1.0×10^{-4} to 3.0×10^{-4} mol l^{-1} and then tended towards stabilisation. A 3.0×10^{-3} mol l^{-1} ammonium monovanadate concentration was selected for posterior experiments.

Aiming at attaining fast sample zone homogenisation, which was in some extent impaired by the high viscosity of the acid solution that restrained mutual sample and reagent zones inter-dispersion, a sample insertion comparison study between binary sampling and single continuous volumes was carried out. Instead of inserting the sample as a unique volume that was sandwiched between two reagent zones, the sample volume was divided into multiple fractions, of few micro-litres each that

were intercalated with multiple equally sized reagent fractions. This scheme created not two but multiple reaction interfaces that affected homogenisation and reaction development in different manners. First of all, unlike sequential injection or merging zones, reaction started immediately after the first small sample aliquot was aspirated into the analytical pathway continuing as the remaining sample was introduced, and not only after the introduction of the whole sample volume or after its merging with a confluent reagent stream. Moreover, the increased contacting area between sample and reagent solutions improved the mixing efficiency and favoured homogenisation. This way, not only the entire sample plug participated uniformly in the reaction from the beginning, which resulted in increased reaction time, but also the dispersion that would be typically necessary to extend the reaction from the boundaries sample/reagent to the central section of sample zone was advantageously reduced. On the other hand, considering that an expeditious strategy to increase peak height is to increase sample volume, sensitivity greatly benefits from the developed approach. In fact, by inserting increased sample volumes it would be problematic to guarantee an adequate excess of reagent owing to insufficient penetration of the reagent zones [15], which occurred only at the reaction interfaces, even considering that this situation was in some extent compensated by using two reagent zones and by reverting the flow direction. With the developed procedure it was possible to increase the sample volume and at the same time to assure an adequate amount of reagent as to guarantee a suitable reaction development.

Finally, during flow-reversal there was not just a reversal of two reaction interfaces but a reversal of multiple adjoining reaction interfaces that interpenetrate and overlap each other in such an extension as to guarantee high and reproducible analytical signals, and thus increased sensitivity, without the need of further dispersion in the second reactor.

In this study, the number and timing of each intercalation cycle defined the final sampling time, which, in addition to the flow rate, determined the inserted sample volume. Since these parameters were strictly controlled a complete and effective control of the sample dispersion was attained. A 10.0 μg ml^{-1} trimipramine solution was injected in the system and a 3.0×10^{-3} mol l^{-1} ammonium monovanadate solution was used as reagent. Carrier solution was sulphuric acid 4.0 mol l^{-1}. Sampling time was programmed in order to attain identical whole sample and reagent volumes for the two sampling approaches. The first results showed that the analytical signals obtained with binary sampling were inferior to those obtained by using continuous sample volumes. These apparently incoherent results could be explained by either the utilisation of the second reactor, which resulted in an excessive residence time where dispersion prevails or insufficient reagent amount (low concentration) that leaded to a dilution effect. Accordingly, the manifold configuration was modified and the second reactor (L_2) placed before detector was removed, remaining only L_1 (Fig. 1). With this modification sample zone homogenisation occurred only within L_1 immediately after the insertion step. In order to diminish the length of sample/reagent zone (and consequently the volume) the binary sampling intercalation sequence was modified by reduction of the reagent intercalation time: instead of 1 s sample/1 s reagent, a sequence consisting on 1 s sample/0.25 s reagent was used. Simultaneously the ammonium monovanadate concentration was increased to 8.9×10^{-3} mol l^{-1}. In these circumstances binary sampling yielded analytical signals higher than those obtained with the undivided sample volume confirming that the efficiency of the sample/reagent mixing and the residence time within L_1 were sufficient to achieve reaction completion after which dispersion prevails. Furthermore, the sampling rate was concomitantly increased while reagent consumption decreased. We have also evaluated the feasibility of using shorter sample intercalation times (0.25, 0.5, 0.75 and 1 s) while maintaining the reagent intercalation time at 0.25 s. Confirming the results obtained in a previous work [16] we verified that the variations in the resulting analytical signals were irrelevant. Considering that the reproducibility attained with an intercalation time of 1 s was higher than the one obtained with shorter times, probably a conse-

quence of the valves operational characteristics, this value was the one selected for posterior experiments.

With this new manifold configuration, and after verifying that the results obtained with the binary sampling approach for ammonium monovanadate effect were appreciably different than those obtained by the single sample volume, the influence of sulphuric acid concentration was re-evaluated. By assaying an increasing number of cycles for an intercalation sequence of 1 s of 20.0 μg ml^{-1} trimipramine solution followed by 0.25 s of 8.9×10^{-3} mol l^{-1} ammonium monovanadate solution it was observed that the analytical signal increased manifestly with the acid concentration until 4.0 mol l^{-1} and then approached stabilisation. A parallel study using single sample volumes matching the binary sampling whole volume (established in terms of identical global sampling time) showed a similar behaviour except that the highest analytical signal was obtained for sulphuric acid 5.0 mol l^{-1}. These results could be explained, as it was previously referred, by the increased sample zone homogenisation provided by binary sampling. Confirming this assumption it was verified that for the same sampling time and for 4.0 mol l^{-1} sulphuric acid the analytical signal obtained with binary sampling was markedly higher than the one obtained with a single sample volume (Fig. 2). Moreover, the reproducibility was greatly enhanced.

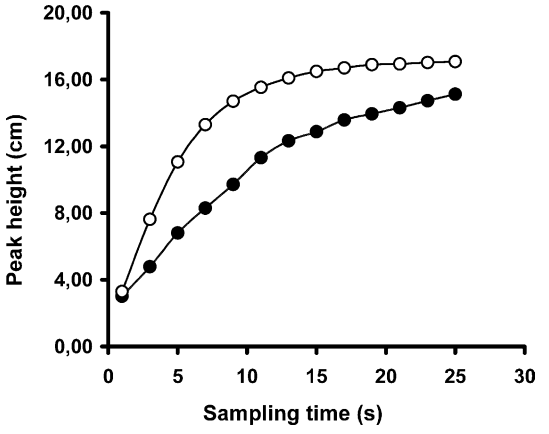

Fig. 2. Analytical signals obtained with increasing sampling times by using distinct sampling strategies: ●—single sample volumes; ○—binary sampling (intercalation sequence consisting on 1 s sample/0.25 s ammonium vanadate 8.9×10^{-3} mol l^{-1}).

Flow rate was evaluated in combination with the binary sampling sequence by using different sample and reagent insertion times that determined for a given flow rate the inserted volumes. A major limitation was the syringe-pump operational characteristics that impaired the maximum available flow rate. Considering that it was equipped with a 5.0 ml syringe and that it was driven by a stepper motor with a full range of 2500 steps (2 μl for each step), a flow rate of 1.5 ml min^{-1} would correspond, approximately, to 750 steps for minute or to a step for each 0.08 s, which approached the syringe-pump maximum operational frequency limiting flow stability.

Aiming to obtain a compromise between sampling rate and sensitivity, which as a result of the fast sample zone homogenisation could be increased by restraining sample dispersion, an intercalation sequence consisting on 11 cycles of 1 s sample/0.25 s ammonium vanadate and a flow rate of 1.5 ml min^{-1} was selected for posterior experiments.

Total insertion times were then 11 s for trimipramine and 3 s for ammonium monovanadate (each insertion cycle begun and ended with a plug of ammonium monovanadate).

An important advantage of the developed procedure is that the reaction products are carried out under positive pressure through the detector, while in most of the cases in multicommutation [17] the pump is placed after the detector being the sample zone aspirated. Alternatively, in some multicommutated flow systems [18] sample transportation by impulsion required the reagents to be re-circulated. The utilisation of a positive pressure reduced the occurrence of air bubbles, as it happened with negative pressures, which could seriously impair detection.

3.1. Analysis of pharmaceutical preparations

After system optimisation, by using a 1.5 ml min^{-1} flow rate and 11 cycles of 1 s sample/0.25 s of 8.9×10^{-3} mol l^{-1} ammonium monovanadate, linear calibration plots between 1.0 and 18.0 μg

ml^{-1} trimipramine were obtained. The analytical curve was represented by $A = 0.7888C + 0.6976$ with a correlation coefficient of 0.9967. In the equation A was the peak height expressed in cm and C was the concentration of trimipramine expressed in mg l^{-1}.

The developed analytical methodology was applied to the determination of trimipramine in pharmaceutical preparations. The obtained results were used to evaluate the system by comparison with trimipramine determination by the reference procedure. Relative deviations (in percentage) between -0.26 and 2.37 were obtained. The relative standard deviation was lower than 1.69% ($n = 10$).

The sample throughput was about 26 samples per hour.

The system proved to be stable and no baseline drift was verified. Table 1 summarised the obtained results.

To assure total reliability of the developed methodology in the determination of trimipramine in pharmaceutical formulations the influence of some compounds commonly used as excipient was investigated. A compound was considered as non-interfering if the analytical signal variation was $\pm$ 3% when compared to the analytical signal obtained in the absence of the referred compound. Results showed that excipients (glucose, sucrose, galactose, lactose, sodium benzoate and magnesium stearate) upon a 100-fold molar ratio regarding trimipramine did not interfere.

4. Conclusions

The obtained results showed that the reaction of trimipramine with ammonium monovanadate constitutes a valuable alternative approach for the determination of trimipramine in pharmaceuticals because it does not require heating or long time exposure and it is not subject to interference from the excipients normally used in pharmaceutical preparations. When implemented in a multicommutated flow system results in a simple, low-cost, fast, sensitive and precise methodology that could be advantageously applied to routine analysis.

The novel homogenisation approach explored in this work, consisting on the combination of binary sampling and flow-reversal, exhibited a very high mixing efficiency that permitted to markedly reduce sample dispersion while assuring a convenient reaction development.

The advantageous operational characteristics of multicommutation, namely the high degree of automation and versatility that it provides, ensure an effective control of the analytical parameters and thus of the chemical reaction and enable a wide range of manipulations of the sample zone without requiring any sort of physical reconfiguration of the flow manifold. Consequently, the scope of the developed methodology could be extended to other chemical reactions or to the determination of other species by performing only minor modifications on the characteristic modular structure of the system. For instance, to insert a new reagent it is only necessary to add a new solenoid valve.

Table 1
Results obtained in the determination of trimipramine in pharmaceutical formulations

Sample	Amount declared (mg/formulation)	Lot analysed	Amount found (mg/formulation) Developed methodology	Reference method	R.D.[a] (%)
Surmontil 25	25	A	24.55 ± 0.17	24.61	−0.26
		B	24.65 ± 0.21	24.56	0.35
		C	25.19 ± 0.27	25.15	0.14
Surmontil 100	100	A	99.52 ± 1.68	98.27	1.27
		B	101.92 ± 1.04	99.56	2.37

[a] Relative deviation, expressed in percentage, of the developed methodology regarding the reference procedure.

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