Determination of Fluoxetine, Fluvoxamine, and Clomipramine in Pharmaceutical Formulations by Capillary Gas Chromatography

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Abstract

A simple and fast capillary gas chromatographic method with flame ionization detection is proposed for the simultaneous determination of fluoxetine, fluvoxamine, and clomipramine without a prederivatization. The reported method is the first one that allows the determination of three selective serotonin reuptake inhibitors. Optimal conditions for the quantitative separation were investigated: column head pressure (80 kPa), injector and detector temperatures (260 and 250°C), time and temperature for the splitless step (0.75 min and 60°C), size of sample (2 µL), and oven temperature program, providing analysis times shorter than 10 min. Aspects such as the stability of the solutions, linearity, accuracy, and precision are examined in order to validate this method. Peak purity and detection and quantitation limits are also assesed using mass selective detection. The scope of the validated method is tested in the analysis of pharmaceutical preparations, with recoveries between 97.5 and 102.5% with regard to their nominal contents.

Introduction

Serotonin (5-HT) has been implicated in the aetiology of many disease states and may be particularly important in mental illnesses such as depression, anxiety, schizophrenia, eating disorders, obsessive compulsive disorder, migraine, panic disorders, bulimia, etc. Indeed, many currently used treatments of these disorders are thought to act by modulating the serotoninergic tone. During the last decade, multiple 5-HT receptor subtypes have been characterized. This has led to the realization of many treatments acting via the serotoninergic system, such as selective serotonin reuptake inhibitors (SSRIs), antidepressants that increase presynaptic 5-HT function.

A group of six antidepressants (citalopram, fluoxetine, fluvoxamine, clomipramine, paroxetine, and sertraline) have been introduced and differ from tricyclic antidepressants (TCA, previously used in the treatment of depression) both in chemical structure and their mechanism of action. SSRIs are comparable to TCA in their clinical efficacy, but due to their favorable pharmacological profile, they are considered to be safe and well-tolerated drugs (1). The SSRIs widely differ in their chemical structure; this explains differences in their metabolism and pharmacokinetics.

In this work, for the first time, the simultaneous determination of three SSRIs, fluoxetine (hydrochloride), fluvoxamine (maleate), and clomipramine (hydrochloride), is accomplished (Figure 1). Furthermore, this becomes a very advantageous method, because it is the first capillary gas chromatographic (GC) method that permits the analysis of SSRIs without a derivatization step.

Most of the studies already carried out on SSRIs have accomplished the separation and determination of fluoxetine and its main metabolite, norfluoxetine, in biological samples (serum, plasma, or tissues) by several techniques such as high-performance liquid chromatography (HPLC)–ultraviolet detection (2–4), GC–electron-capture detection (ECD) (5) or GC–mass-selective detection (MSD) (6). Other works include the quantitation of enantiomeric forms of both fluoxetine and norfluoxetine by HPLC (7) and GC–ECD (8) techniques. It must be emphasized that all the already existing GC methods are based on previous derivatization reactions.

Determination of fluvoxamine has rarely been achieved until now; GC has not been used to analyze fluvoxamine in the already reported papers; the only two works found approach its measure by HPLC techniques in different samples such as tablet formulations, human serum, etc. (9,10).

The aim of the present work is to propose an easy and fast GC–flame ionization detection (FID) method that permits the determination of fluoxetine, fluvoxamine, and clomipramine in any of their pharmaceutical preparations and that could be a valuable alternative to the existing official methods established by the *European Pharmacopeia* (11). Also, this method has been succesfully applied to all the existing pharmaceutical preparations containing any of the three SSRIs studied.

Experimental

Reagents

Methanol (HPLC grade) was purchased from PANREAC (Madrid, Spain). Fluoxetine (hydrochloride) (FLX), fluvoxamine (maleate) (FLV), and clomipramine (hydrochloride) (CLO) were supplied by TOCRIS (Bristol, U.K.). Standard solutions (200 mg/L) were prepared in methanol and stored in a refrigerator at 4°C. Working standard solutions were prepared daily by diluting the stock standard solutions with methanol.

Instruments

The equipment used was a Hewlett-Packard (Palo Alto, CA) 5980 series II GC provided with a 6890 autosampler, a split/splitless injector, flame ionization and 5971 series mass-selective detectors, and HPG1701AA MS Chemstation software (12).

The column was an HP-5 (5% phenyl-methylsilicone, 15 m × 0.25-mm i.d, 0.25-µm film thickness) supplied by Hewlett-Packard.

Operating conditions

For the separation procedure, the instrumental parameters were as follows: carrier gas, helium; whole flow, 50 mL/min; column head pressure, 80 kPa; flow rate, 1.2 mL/min; injector temperature, 260°C; FID temperature, 250°C; injected volume, 2 µL; oven temperature program, 60°C for 0.75 min (splitless step), then programmed to 182°C at 70°C/min and held for 0.5 min, then to 185°C at 1°C/min and held for 0.5 min, and finally to 250°C at 70°C/min and held there for 5 min.

Because all of the analyzed pharmaceutical preparations only contained one of the studied SSRIs, any of the other two antidepressants could be used as an internal standard to achieve quantitation following the internal normalization criterion (13) in these pharmaceutical applications.

Duplicate injections of the solutions were performed, and average relative peak areas were used for quantitation (using in all analyses a content of 10 mg/L of the SSRI selected as internal standard in each quantitation).

Although the determination of the three SSRIs and their application in pharmaceutical preparations was mainly developed with an FID, some specific aspects of validation such as peak purity and detection and quantitation limits were also assesed with an MSD working in SCAN and selected ion monitoring (SIM) mode, respectively. For these assays, the general instrument conditions of MS detection were as follows: interface temperature, 280°C; ionization energy, 70eV; EM voltage, 1800 V; in SCAN mode, mass range = 35–350 amu, scan rate = 2.39 scan/s, and solvent delay = 2.5 min; particularly in SIM mode, scan rate = 5.88 cycles/s, dwell time = 40 msec, and solvent delay = 4.5 min; monitored selected ions were fluoxetine (*m/z* 44, 104, 309), fluvoxamine (*m/z* 43, 187, 276), and clomipramine (*m/z* 58, 85, 269).

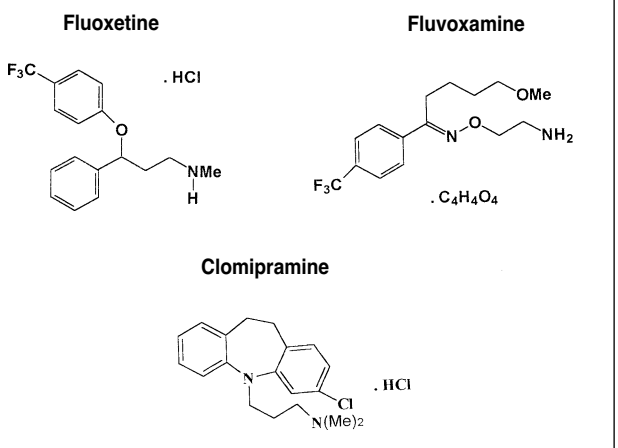

Figure 1. Structures of studied SSRIs.

Sample preparation

Duplicate test and standard solutions were prepared as follows.

Standard solutions

Approximately 20 mg of FLX, FLV, or CLO was weighed accurately, approximately 50 mL of methanol was added, and this was shaken by means of a magnetic stirrer for 5 min. It was then transferred to a 100-mL volumetric flask and diluted with methanol to 100 mL. From these stock solutions, measured aliquots were taken and diluted with methanol to give different final concentrations of fluoxetine, fluvoxamine, or clomipramine.

Test solutions

Capsules and envelopes. The content of a capsule or an envelope was placed in a beaker, approximately 90 mL of methanol was added, and this was shaken mechanically for 5 min. The suspension was placed in a 100-mL volumetric flask, diluted with methanol to the mark, and centrifuged at 5000 rpm for 5 min. An aliquot (0.5 mL) from the supernatant was diluted 1:20 (v/v) with methanol to give a final concentration of approximately 10 mg/L of fluoxetine, and a known amount of stock solution of clomipramine was added to obtain a content of 10 mg/L (internal standard).

Tablets. A tablet was weighed and ground in a mortar. The solid was transferred into a beaker, and approximately 90 mL of methanol was added. This was mechanically shaken for 5 min. The suspension was placed into a 100-mL volumetric flask, diluted with methanol to the mark, and centrifuged at 5000 rpm for 5 min. An aliquot from the supernatant was diluted 1:20 (v/v) with methanol to give a final concentration of approximately 10 mg/L of fluvoxamine, fluoxetine, or clomipramine, and a known amount of stock solution of clomipramine (10 mg/L) (to determine fluoxetine and fluvoxamine) or a known amount of stock solution of fluoxetine (10 mg/L) was added as an internal standard (to quantitate clomipramine).

Solutions. Five milliliters of pharmaceutical solution was diluted with methanol to the mark in a 100-mL volumetric flask. An aliquot of 0.5 mL from this solution was diluted 1:20 (v/v) with methanol to give a final concentration of approximately 10 mg/L of fluoxetine, and a known amount of stock solution of clomipramine (10 mg/L) was added as an internal standard.

Results and Discussion

Optimization of capillary GC procedure

Head pressure column

The effects of the head pressure column are explored by the van Deemter equation, which examines the efficiency of the column (or more accurately, of the entire system) as a function of the average linear carrier gas velocity (μ).

Column efficiencies are expressed in terms of *H* (equivalent height of theoretical plate). Figure 2 shows the obtained van Deemter curves for the three compounds when the efficiency of the column (*H*) is represented versus μ. This parameter was varied between 11.0 cm/s (20 kPa head pressure) and 71.5 cm/s (150 kPa), a value of 43.6 cm/s (80 kPa) being selected as optimum because it provided good efficacy without excessively decreasing the carrier gas velocity (in order to avoid unnecesarily extending the time of analysis). The selected head pressure involves a gas flow through the column of 1.2 mL/min.

Temperature injector

The temperature in the injection port must be maintained high enough to avoid condensation of samples but not so much as to produce the breakdown of thermally unstable components, which may form artifacts at high temperatures. Thus, the temperature of the injection port was increased from 200 to 300°C, a value of 260°C selected as optimum in order to obtain a suitable signal for the three components.

Splitless injection parameters

This method is useful for very dilute solutions. When splitless injection is used, the column is overloaded with the solvent. For this reason, the temperature at the top of the column is kept low (10–20°C below the boiling point of the solvent) so that the low-volatility components and the solvent condense. This condensation causes the components to be focused. The method is not recommended for volatile components, because these are eluted from the column with the solvent. Care must be taken in this procedure to prevent the injector from being overloaded by the quantity of injected sample, because this can be a source of contamination in subsequent analysis. For this reason, the parameters to be optimized are the time and temperature at which the splitless step takes place.

Splitless time. The times during which the focusing step occurs must be controlled, because if the amount of sample applied is too large (splitless time too long), the separating capacity of the capillary column is reduced due to overloading, resulting in an inefficient separation. Figure 3 shows the effect of different splitless times on the three SSRI areas. As can be seen in this figure, splitless times longer than 0.75 min produce a decrease in signal areas for fluoxetine and fluvoxamine and longer analysis times. A value of 0.75 min was chosen as optimum for the separation procedure, because it provided a balance between a higher sensitivity for the three compounds and shorter analysis times.

Splitless temperature. The effect of temperature at which the condensation step takes place was studied over the signal and retention times in a range from 40 to 80°C. As expected, applications of lower temperatures led to higher peak areas but increased analysis times and baseline aberrations. Therefore, 60°C was selected for this study, because it again provided a balance among higher sensitivity, shorter analysis times, and lower baseline contamination.

Temperature FID

The temperature in the FID must be high enough to avoid the condensation of samples, but if this temperature is excessively high, it can damage the detector and increase the noise level and baseline aberrations of the signal. Several FID temperatures were checked (225, 250, and 275°C), and 250°C was selected as optimum for the reasons cited before.

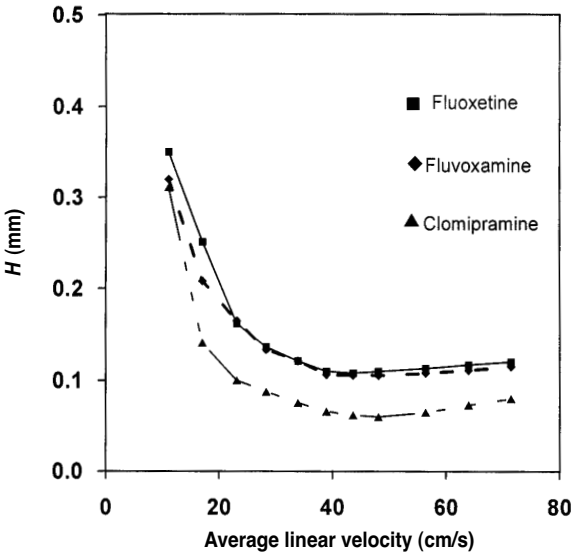

Figure 2. Van Deemter curves for the three SSRIs when *H* is represented versus μ.

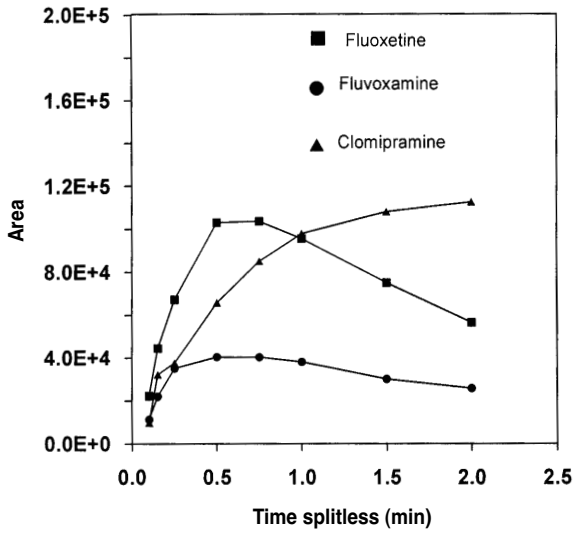

Figure 3. Influence of splitless time on the three SSRI's peak areas.

Volume of injected sample

Different aliquots from 1 to 3 µL were injected into the GC and observed for a linear relationship between injected volumes and peak areas in the tested range for the three SSRIs. An intermediate volume of 2 µL was selected in order to obtain good sensitivity and low baseline aberrations.

Table I. Capillary GC Selected Procedure

Experimental conditions	
Carrier gas	helium, 43.6 cm/s, 80 kPa, 1.2 mL/min gas flow
Oven to	60°C (held 0.75 min) to 182°C at 70°C/min (held 0.5 min), 185°C at 1°C/min (held 0.5 min), to 250°C at 70°C/min (held 5 min)
Injection	splitless, 2 µL, 260°C
Detector	FID, 250°C; MSD mode, SIM

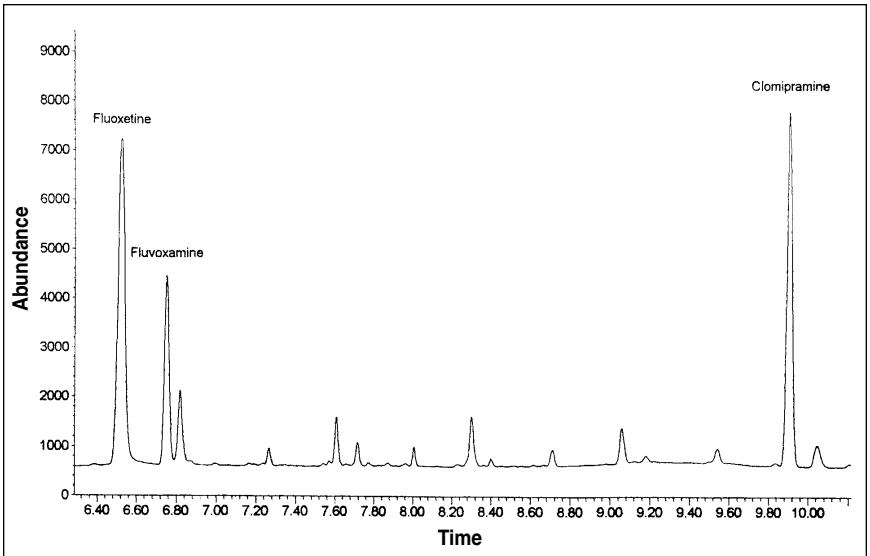

Figure 4. Capillary gas chromatogram for a standard mixture of fluoxetine, fluvoxamine, and clomipramine under the selected experimental conditions.

Table II. Linearity (n = 7), LOD, and LOQ of the SSRIs Studied

		FLX	FLV	CLO
Equation*		$y = -0.041 + 0.095x$	$y = -0.027 + 0.056x$	$y = 0.051 + 0.119x$
Coefficient of correlation		0.9998	0.9990	0.9999
SD* slope		7.3×10^{-4}	1.1×10^{-3}	7.7×10^{-4}
SD† intercept		2.7×10^{-2}	4.0×10^{-2}	2.9×10^{-2}
LOD (µg/L)	FID	9.75	101.0	38.5
	MSD	11.0	18.0	7.73
LOQ (µg/L)	FID	26.5	248.0	66.0
	MSD	35.0	42.0	18.5

* Concentration (x) versus relative peak area (y).
† SD, standard deviation.

Oven temperature program

Under injection and detection conditions previously selected, several assays were performed to find a temperature program that provided the separation of the three SSRIs. One of the best options was the following program, because it supplied $Rs \geq 1.5$ in an analysis time less than 10 min: 60°C for 0.75 min (splitless step), to 182°C at 70°C/min and held 0.5 min, to 185°C at 1°C/min and held 0.5 min, to 250°C at 70°C/min and held 5 min.

In Figure 4, the chromatogram obtained for a mixture of the three SSRIs when this oven temperature program was applied is shown. In Table I, the optimized conditions for the capillary GC method are summarized.

Validation of the selected GC procedure

Stability of the solutions

Although this test is often considered part of the ruggedness of the procedure, it should be carried out at the beginning of the procedure validation because it determines the validity of the data of the other tests.

The stability of the standard and test solutions of fluvoxamine, fluoxetine, and clomipramine was determined by comparing the response factors (concentration/average peak areas) of duplicate solutions stored at room temperature and 4°C, in darkness and light, with those of freshly prepared duplicate solutions. Less than a 0.2% concentration difference was found between the solution freshly prepared and those aged for 7 days. Furthermore, the absorption spectra of these solutions were found to be unchanged for this period. The solutions can therefore be used within this period without the results being affected.

Linearity

Detector response measured for the studied antidepressents was linearly correlated with the sample concentration injected over a range of 0.5–80 mg/L for all the compounds studied.

The linearity was determined from repeated injections at seven different concentrations of each antidepressant. The linear regression equations for these SSRIs obtained under our conditions are summarized in Table II. The correlations between the concentrations of each SSRI and its relative peak area are close to 1.0 in all the cases in the concentration range assayed.

Accuracy

In order to check the accuracy of the proposed method, several synthetic mixtures containing the three SSRIs in different ratios were prepared and analyzed using the GC procedure described. The results obtained using the relative peak area are

summarized in Table III. As can be seen in all cases, good recoveries (%) were obtained.

Precision

The precision of the proposed method is expressed in terms of relative standard deviation (RSD).

In order to check the precision of the chromatographic procedure, eight injections of a standard of 10 mg/L of fluoxetine, fluvoxamine, and clomipramine were carried out sequentially. The precision of the retention time and relative peak areas were excellent, with RSD (%) values (n = 8) of 0.25, 0.12, and 0.05% for migration time and 2.28, 1.31, and 1.19 for relative peak area for fluoxetine, fluvoxamine, and clomipramine, respectively.

Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated by measuring ten blanks using the maximal sensitivity allowed by the system and calculating the standard deviation (SD) of this response. LOD was estimated by multiplying the SD by a factor of 3. The LOQ was defined as 10 times the SD.

Although the sensitivity provided by the FID is adequate to carry out the analysis of the studied antidepressants content in the pharmaceutical formulations, in order to extend the scope of the method to samples such as plasma, urine, and serum (with very low levels of SSRIs), this study was also performed using an MSD operating in SIM mode.

Taking into account the mass spectra of fluoxetine, fluvoxamine, and clomipramine (Figure 5), the following selected ions were monitored in SIM acquisition: fluoxetine, *m/z* 44, 104, and 309; fluvoxamine, *m/z* 43, 187, and 276; clomipramine, *m/z* 58, 85, and 269.

Under these conditions, the LODs and LOQs obtained using the two detectors are summarized in Table II. The LOQ was subsequently validated separately by the analysis of six standards prepared at their respective concentrations for fluoxetine, fluvoxamine, and clomipramine.

Applications

To demonstrate the usefulness of the developed GC procedure, the method was applied to the determination of the analytes in any of their pharmaceutical preparations commercially available in Spain, which contain an antidepressant with other components.

Table III. Recoveries (%) for Different Synthetic Mixtures of the Studied SSRIs

Composition of mixtures (mg/L)			Recoveries (%)*		
FLX	FLV	CLO	FLX	FLV	CLO
2.50	5.00	11.25	113.5	100.8	103.5
10.50	2.50	7.50	104.7	94.1	101.1
5.00	10.00	3.75	102.4	104.9	105.1
10.50	10.00	11.25	103.1	105.1	101.2
12.00	5.00	15.00	101.1	103.6	97.9
15.40	12.50	7.50	95.4	103.1	101.6
5.00	15.00	18.75	105.8	104.7	99.7

* Mean of two determinations.

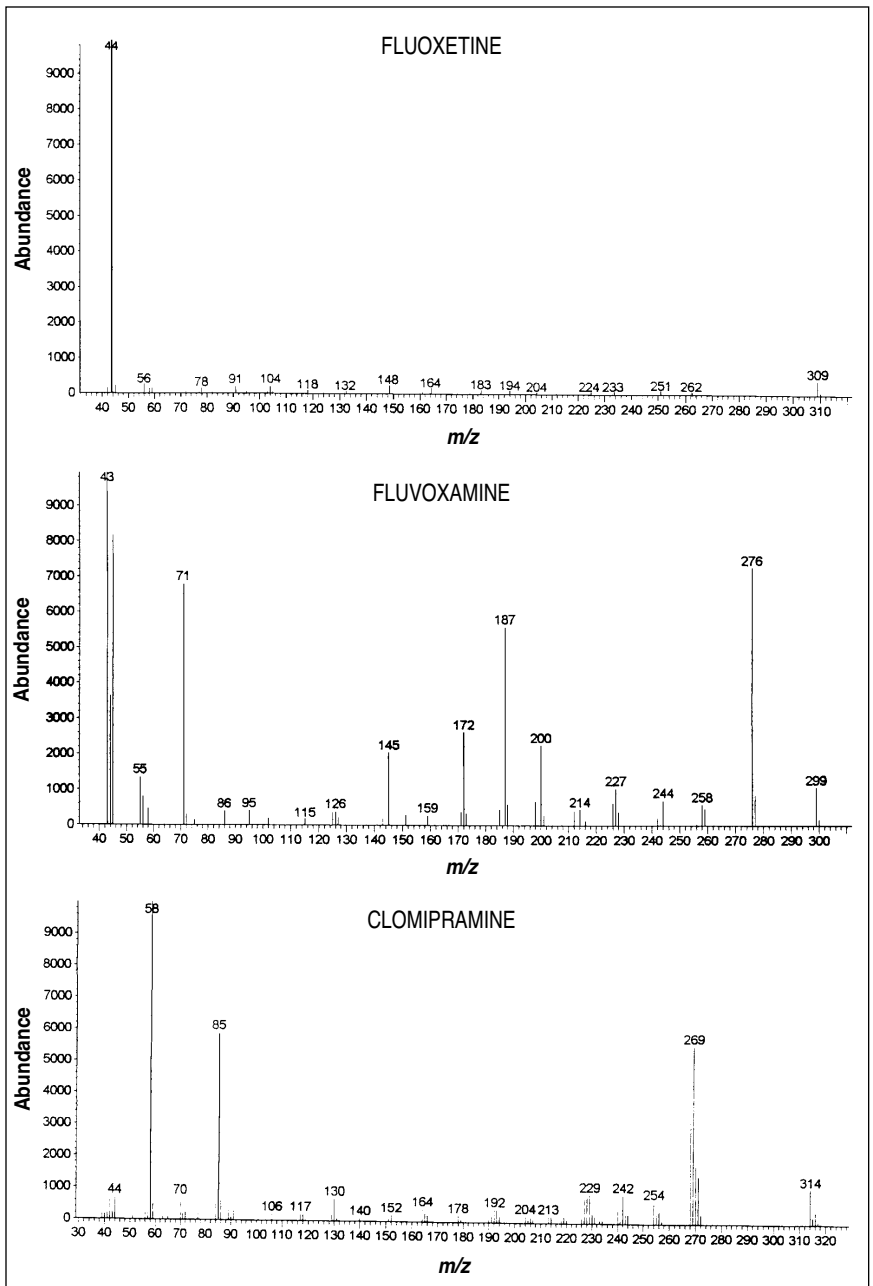

Figure 5. Mass spectra of the three studied SSRIs.

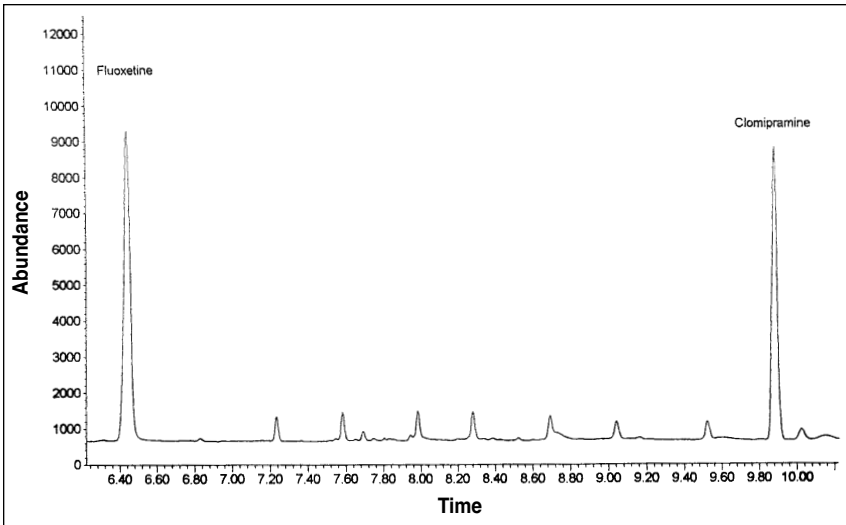

Figure 6. Capillary gas chromatogram for a sample of Prozac capsules (fluoxetine) using clomipramine as an internal standard.

Preparation of the samples is described in the Experimental section. As in any separation technique, coelution of peaks is possible in capillary GC. Therefore, it is useful to investigate the purity of separated peaks. Peak purity was checked for all of the analyzed pharmaceutical preparations by the use of an MSD working in SCAN mode. Analyses of peak purity were performed by means of HPG 1701AA MS Chemstation software (Hewlett-Packard).

As an example, Figure 6 shows a chromatogram of one of the analyzed pharmaceutical preparations (Prozac capsules).

The determination of each SSRI's content in the pharmaceutical formulations was carried out in triplicate. As can be observed in Table IV, the results were reproducible, and the recoveries were in the range of 97.5–102.5% of the declared values by manufacturers.

Table IV. Analysis of Pharmaceutical Preparations

Preparation (presentation)	Declared	Found ± s (mg)*
Adofen, 20 mg (envelope)†	Fluoxetine (DCI) (hydrochloride), sodium sacharin 10 mg, anisette and peppermint flavor, manithol, sorbithol	19.50 ± 0.12
Adofen, 20 mg (tablets)†	Fluoxetine (DCI) (hydrochloride), cellulose, manithol, sorbithol, anisette and peppermint flavor, silica colloidal, starch of maize, sodium fumarate of estearile, polividone	19.75 ± 0.19
Adofen, 20 mg (capsules)†	Fluoxetine (DCI) (hydrochloride), starch, fluid silicone	19.70 ± 0.85
Reneuron, 20 mg (envelope)‡	Fluoxetine (DCI) (hydrochloride), sodium sacharin 10 mg, manithol, sorbithol, peppermint and anisette flavor, c.s.	20.20 ± 0.45
Reneuron, 20 mg (solution)‡	Fluoxetine (DCI) (hydrochloride), sacharose 3 g, benzoic acid, glicerine, peppermint, purified water	20.05 ± 0.22
Reneuron, 20 mg (tablets)‡	Fluoxetine (DCI) (hydrochloride), cellulose, manithol, sorbithol, anisette and peppermint flavor, silica colloidal, starch of maize, sodium fumarate of estearile, polividone	19.50 ± 0.39
Reneuron, 20 mg (capsules)‡	Fluoxetine (DCI) (hydrochloride), starch, fluid silicone, c.s.	21.50 ± 0.68
Prozac, 20 mg (envelope)§	Fluoxetine (DCI) (hydrochloride), sodium sacharin 10 mg, manithol, sorbithol, peppermint and anisette flavor, c.s.	19.50 ± 0.29
Prozac, 20 mg (solution)§	Fluoxetine (DCI) (hydrochloride), sacharose 3 g, benzoic acid, glicerine, peppermint, purified water	19.50 ± 0.58
Prozac, 20 mg (tablets)§	Fluoxetine (DCI) (hydrochloride), cellulose, manithol, sorbithol, anisette and peppermint flavor, silica colloidal, starch of maize, sodium fumarate of estearile, polividone	20.50 ± 0.39
Prozac, 20 mg (capsules)§	Fluoxetine (DCI) (hydrochloride), starch, fluid silicone, c.s.	20.0 ± 0.68
Dumirox, 100 mg (tablets)‖	Fluvoxamine (maleate), manithol, starch of maize, sodium estearasefumarate, pregelatinized starch, silica coloidal anhidre, methylhydroxipropylcelulose, polyethylenglycol 6000, talcum powder, titanium dioxide	101.67 ± 0.50
Anafranil, 75 mg (tablets)#	Clomipramine (DCI) hydrochloride, silicic colloidal acid, calcium phosphate dibasic, calcium estearate, hydroxipropylmethylcellulose, red iron oxide, castor oil, talcum, titanium dioxide	76.02 ± 0.21
Anafranil, 25 mg (ampules)**	Clomipramine (DCI) hydrochloride, glycerine, water for injection	24.97 ± 0.37

* Mean value ± SD (n = 3).
† Obtained from Ferrer International.
‡ Obtained from Juste S.A.Q.F.
§ Obtained from Dista S.A.
‖ Obtained from Duphar S.A.
Obtained from Novartis Farma S.p.A.
** Obtained from Novartis Pharma S.A.

Conclusion

For the first time, a capillary GC method is reported that combines the possibility of determining three SSRIs without the need of a previous derivation step. This method is much faster and easier than those previously found in the literature and permits the successful quantitation of any of the three studied SSRIs in their pharmaceutical preparation without interferences. The scope of the proposed method was extended to samples with very low levels of SSRIs by the use of an MSD working in SIM mode.

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