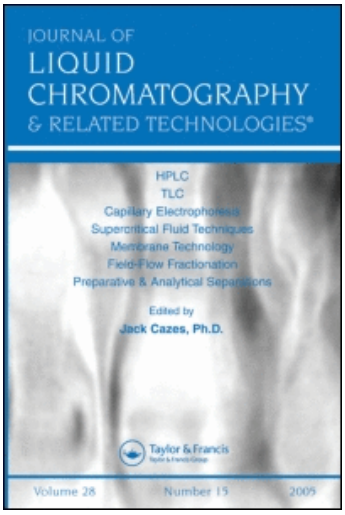

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T. Gondová[a]; D. Halamová[a]; K. Špacayová[b]
[a] Department of Analytical Chemistry, Faculty of Science, P.J. Šafárik University, Košice, Slovak Republic [b] Health Care Surveillance Authority, Patological-Anatomical and Medico-Judicial Workplace of Regional Office Košice, Košice, Slovak Republic

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Simultaneous Analysis of New Antidepressants by Densitometric Thin-Layer Chromatography

T. Gondová,[1] D. Halamová,[1] and K. Špacayová[2]

[1]Department of Analytical Chemistry, Faculty of Science, P.J. Šafárik University, Košice, Slovak Republic
[2]Health Care Surveillance Authority, Patological-Anatomical and Medico-Judicial Workplace of Regional Office Košice, Košice, Slovak Republic

Abstract: A simple and rapid TLC method using densitometric detection was developed for the simultaneous analysis of four SSRI antidepressants: citalopram, sertraline, fluoxetine, and fluvoxamine. The analytes were separated on silica gel 60 F_{254s} TLC plates using a mobile phase composed of acetone-benzene-ammonia (50:45:5, v/v/v). Densitometric detection and quantification were carried out in the reflectance mode at 240 nm. The calibration curve was linear in the range of 500–5000 ng per spot for all analyzed compounds. The limit of detection was 40 ng per spot for citalopram and 50 ng per spot for fluoxetine, fluvoxamine, and sertraline, respectively. Statistical analysis proves that the method is both precise and accurate. The proposed method was successfully applied for the determination of citalopram and fluoxetine in their pharmaceutical formulations, with recoveries ranging from 98.7–101.9% of the labeled amount of the compounds. Finally, the reported method was also applied to the analysis of antidepressants in real biological samples.

Keywords: Citalopram, Fluoxetine, Fluvoxamine, Pharmaceutical formulations, Sertraline, TLC

Correspondence: T. Gondová, Department of Analytical Chemistry, Faculty of Science, P.J. Šafárik University, Moyzesova 11, Košice SK-040 01 Slovak Republic. E-mail: tatana.gondova@upjs.sk

INTRODUCTION

Depression is a common mental disorder affecting an increasingly large portion of the worldwide population of all ages. It is, in fact, becoming a significant modern disease of the third millenium. In some cases it is accompanied by such suffering that it can lead to suicidal behaviour.[1]

Selective serotonin reuptake inhibitors (SSRI) represent a new generation of antidepressants being used for the treatment of depressive disorders and other indications. These substances can regulate the concentration of serotonin (a neurotransmitter affecting mood, cognition, sleep, appetite, hormone secretion, etc.) in the central nervous system. Over the past decade, SSRI, which include fluoxetine, fluvoxamine, sertraline, citalopram, and paroxetine, have become the most widely used group of antidepressants, as they are considered to be both safe and well tolerated.[2,3]

The development of fast, selective, and reliable methods that enable the screening and determination of these substances could be of great interest in research, pharmaceutical analysis, therapeutic drug monitoring, as well as in toxicological and forensic analysis in cases of antidepressant intoxication.

Most of these methods have been developed individually for each drug as described in the review.[4] On the other hand, methods which enable the simultaneous analysis of several SSRI antidepressants in a single step are useful for practical and economic reasons.

Several methods for the simultaneous analysis of selective serotonin reuptake inhibitors in biological samples or in pharmaceutical formulations have been developed. The majority of these methods are based on high performance liquid chromatography (HPLC),[5–7] gas chromatography (GC), GC with mass spectrometry (MS),[8,9] or thin-layer chromatography (TLC).[10] Recently, capillary electrophoresis has also been used in the analysis of SSRI antidepressants in pharmaceutical products and biological fluids.[11,12] Although TLC is less sensitive when compared with GC/MS or HPLC, it offers several advantages, including simplicity of use, the ability to repeat detection and quantification with changed parameters, cost effectiveness, as many samples can be analyzed on a single plate with a low amount of solvent, shorter analysis time, and minimal sample cleanup. On the other hand, the development of HPTLC instruments for automated sample application and densitometric evaluation in situ has made it possible to obtain results that are comparable with those obtained by HPLC.[13]

Only a few papers in the literature have considered the simultaneous analysis of SSRI antidepressants using thin-layer chromatography.

Misztal and Skibinski[10] reported the simultaneous analysis of six new antidepressants using reversed and normal phase TLC. The drugs

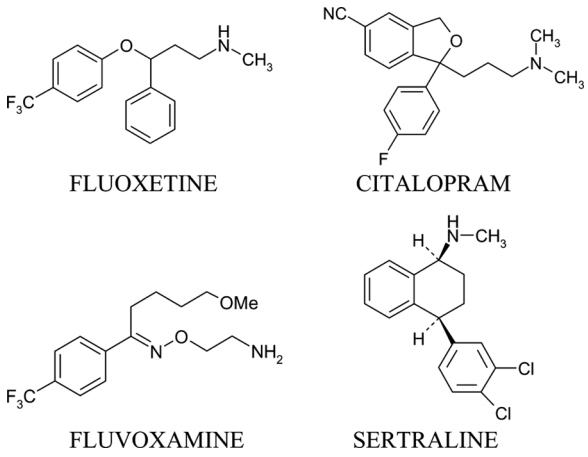

Figure 1. Chemical structures of the studied antidepressants.

were separated on silica gel GF_{254}TLC plates using a mobile phase consisting of benzene-acetone-etanol-ammonia $(9 + 7 + 2 + 1, v/v)$. The limit of detection was 150 ng per spot for citalopram, fluoxetine, and fluvoxamine. Determinations of fluoxetine and paroxetine,[14] as well as fluvoxamine and moclobemide[15] in their pharmaceutical formulations using densitometric and videodensitometric TLC methods under already mentioned conditions, have been also described.

This paper presents a simple and fast densitometric thin-layer chromatographic method, which permits the simultaneous analysis of the four most commonly prescribed SSRI antidepressants: fluoxetine (FLX), citalopram (CIT), fluvoxamine (FLV), and sertraline (SER) (Figure 1). The proposed method was successfully applied to the quantification of citalopram and fluoxetine in their commercially available pharmaceutical form. The method was also applied to the analysis of real biological samples from patients undergoing citalopram and fluoxetine treatment and also in one forensic case.

EXPERIMENTAL

Chemicals and Materials

Methanol, acetone, benzene, and ammonia were obtained from Lachema (Brno, Czech Republic). All chemicals were of analytical grade quality and redistilled deionized water was used.

Fluvoxamine (maleate), fluoxetine (hydrochloride), sertraline (hydrochloride), and citalopram (hydrobromide) were supplied by Slovakofarma (Hlohovec, Slovak Republic).

The following representative commercially available pharmaceuticals were used for the applications: Magrilan (20 mg of fluoxetine per capsule, Medochemie Ltd., Cyprus), Floxet (20 mg of fluoxetine per capsule, Egis Pharmaceuticals Ltd., Hungary), Citalec (20 mg of citalopram per tablet, Zentiva, Czech Republic), and Cipralex (10 mg of escitalopram per tablet, Lundbeck, Denmark).

Chromatographic silica gel 60 F_{254} precoated glass plates were obtained from Merck (Darmstadt, Germany) and Bond Elute Certify (130 mg, 3 mL) phase extraction cartridges supplied by Varian (Middelburg, Netherlands) were employed.

Instrumentation and Chromatographic Conditions

Chromatographic analysis was performed on silica gel 60 F_{254s} glass TLC plates 10 cm × 10 cm. The samples were spotted using a 5 μL microsyringe (Hamilton, Switzerland). The plates were developed at room temperature to a distance of 80 mm in a vertical trough glass developing chamber (Labora, Bratislava, Slovak Republic), with the mobile phase containing a mixture of acetone-benzene-25% ammonia (50:45:5, v/v/v). Before development, the chamber was saturated with the mobile phase for 15 min. The spots were then detected under a UV lamp (254 nm) and evaluated densitometrically at 240 nm with a Shimadzu CS-930 dual wavelength TLC scanner (Kyoto, Japan) used in the reflectance absorbance mode. The peak areas served for quantitative evaluation.

SPE assays were performed on a Vac-Elut vacuum manifold station purchased from Varian (Middelburg, Netherlands).

Standard Solutions, Calibration

Stock standard solutions of antidepressants (1 mg/mL) were prepared separately in methanol and stored at +4°C. Working standard solutions were prepared daily by diluting stock solution with methanol for each antidepressant to yield concentrations of 100, 200, 400, 600, 800, and 1000 μg/mL. These concentrations were used to construct calibration curves. Five microliters of each solution were applied using a 5 μL microsyringe on the TLC plate.

Calibration curves were established over the range of 500–5000 ng per spot by plotting peak areas versus concentration and the regression equations were calculated (Table 1).

Table 1. Regression parameters of calibration curves for the four SSRI studied

Compound	Intercept (a)	Slope (b)	Correlation (r)
Fluoxetine	5471	53.60	0.9982
Citalopram	−8374	448.26	0.9985
Fluvoxamine	20200	149.19	0.9911
Sertraline	6054	81.56	0.9981

Assay Validation

The proposed TLC method for assay of CIT and FLX in pharmaceuticals was validated for the following parameters: linearity, limit of detection and quantification, precision, accuracy, and selectivity.

Linearity was determined from three replicate spottings at six different concentration levels for each antidepressant. Six-point calibration curves were constructed by plotting the peak areas against the corresponding concentrations of the analytes by means of the least-square method in the range of 500–5000 ng per spot. The limit of detection (LOD) was established at a signal-to-noise ratio (S/N) of 3, while the limit of quantification (LOQ) was established at an S/N of 10.

The precision of the developed method was expressed as a percentage of relative standard deviation (% RSD) for repeatability (intra-day precision) and intermediate precision (inter-day precision) at three concentration levels of CIT and FLX standards within the calibration curve range. Repeatability of a sample application and measurement of the peak area were determined on the same day by the repeated application ($n = 6$) of standards solutions, while intermediate precision was evaluated by comparing the assays for three different days.

The accuracy of the developed method was studied through the percentage of recovery of known amounts of CIT or FLX standards added to solutions of the corresponding commercial product within the linearity range. The analyzed samples were spiked with an extra 100 and 150% of standard citalopram or fluoxetine solution. The recovery was calculated by comparing the peak areas obtained with those obtained from pure standards at the same concentrations. All measurements were performed in triplicate.

The selectivity of the method was studied in relation to possible matrix interferences in pharmaceuticals.

Analysis of Pharmaceuticals

Sample solutions of commercial citalopram and fluoxetine drug formulations were prepared as follows: ten tablets of citalopram or ten

capsules of fluoxetine were weighed and finely pulverized. A portion of the powder corresponding to 20 mg of antidepressant was accurately weighed, quantitatively transferred into a 25 mL volumetric flask, and dissolved in methanol for 10 minutes with the aid of sonication. The solution was left to sediment. An appropriate volume of the supernatant was diluted with methanol, so that the final concentration of each antidepressant was within the working range of the calibration curve and subjected to TLC analysis according to the procedure described above. This procedure was repeated in triplicate for each antidepressant.

Analysis of Biological Samples

The urine and blood samples used originated from patients undergoing CIT and FLX treatment and from one forensic case (SER). Antidepressants were isolated and preconcentrated from biological samples using liquid-liquid extraction (urine) or solid phase extraction (serum, blood) prior to TLC analysis.

Urine

A 10 mL urine sample was acidified with 0.25 M hydrochloric acid solution to pH 3–4. Extraction of sertraline was performed with diethyl ether (2×60 mL). After the first extraction, the aqueous phase was alkalized with sodium carbonate to pH 10–11 then extracted with diethyl ether. The diethyl ether extracts were combined and the ether was evaporated to dryness in a water-bath at 60°C. The residue was dissolved in methanol and analyzed.

Serum, Blood

To 1 mL samples of serum (or blood), 4 mL of potassium phosphate buffer (pH 6, 100 mM) were added and the pH adjusted to 6 by adding 1M KOH.

Solid phase extraction of citalopram (or fluoxetine) was performed on a Vac-Elute apparatus using Bond Elute Certify cartridges (130 mg, 3mL). The cartridge was conditioned with 3 mL of methanol, followed by 3 mL of deionized water and 1 mL of potassium phosphate buffer (pH 6, 100 mM), prior to application of the sample. After passing the sample, the cartridge was washed with deionized water (3 mL), 1 M acetic acid (1 mL), and methanol (3 mL). The cartridge was dried under a vacuum for 5 min. Elution was carried out using 3 mL of mixture

consisting of dichloromethane-2-propanol-ammonia (78:20:2, v/v/v) at a flow rate of 1mL/min. After evaporation using a flow of nitrogen at 40°C, the residue was dissolved in methanol and analyzed.

RESULTS AND DISCUSSION

The aim of this work was to develop a simple and fast TLC densitometric method for the simultaneous analysis of four antidepressants, fluoxetine, fluvoxamine, citalopram, and sertraline. TLC separation was performed in normal phase mode using silica gel TLC plates. A densitometric technique was used for in-situ detection and determination of antidepressants based on peak areas. A wavelength of 240 nm was selected after acquiring UV spectra for all of the studied analytes.

To optimize TLC separation, several mobile phases of various polarities were tried. The best separation of the studied compounds was obtained with a mobile phase consisting of acetone-benzene-25% ammonia (50:45:5, v/v/v) at room temperature. Under optimized chromatographic conditions, the average R_F values obtained were 0.28 for fluoxetine, 0.44 for citalopram, 0.56 for fluvoxamine, and 0.68 for sertraline (with a standard deviation less than 0.02 in all cases). The baseline separation was noted among all the analytes with a very good resolution. A representative TLC chromatogram of antidepressants is shown in Figure 2.

Method Validation

Linearity

Under optimum experimental conditions, linear correlation between peak area and concentration was found in the range of 500–5000 ng per spot for each antidepressant. Characteristic parameters for corresponding regression equations are presented in Table 1. Correlation coefficients were found to be more than 0.998 for all the drugs (except the 0.991 for fluvoxamine), confirming good linearity for all calibration curves.

Limit of Detection, Limit of Quantitation

Limit of detection (LOD), which is defined as the lowest concentration which can be detected, was estimated as three times the signal to baseline noise ratio. The limit of quantification (LOQ) was estimated as 10 times the signal-to-noise ratio. Under the experimental conditions described,

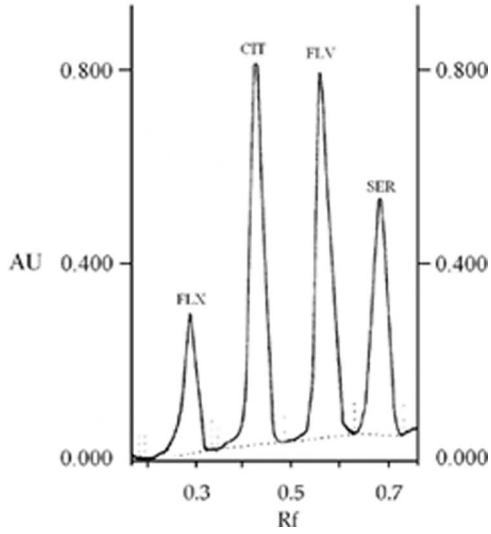

Figure 2. Representative TLC chromatogram of FLX, CIT, FLV, and SER mixture at 240 nm with acetone-benzene-25% ammonia (50:45:5, v/v/v) mobile phase, sample concentration; 0.8 mg/mL each analyte.

the limit of detection was found to be 40 and 50 ng per spot and the limit of quantification was found to be 130 and 160 ng per spot for citalopram and fluoxetine, respectively.

Precision

The precision of the proposed method is expressed in terms of relative standard deviation (RSD). Precision data on the intra- and inter-day variation for three different concentration levels of citalopram and fluoxetine are summarized in Table 2. Intra-assay precision was investigated by six replicate spottings of standard solutions of each analyte at three different concentrations within the calibration curve range. The %RSD values were within the range of 0.52–0.87% and 1.34–1.84% for citalopram and fluoxetine, respectively. Inter-day precision for the analyses conducted on three consecutive days were found to be less than 1.8% and 2.5% for CIT and FLX, respectively.

Accuracy

Recoveries of citalopram and fluoxetine were obtained by spiking the analyzed samples of the pharmaceuticals with 100 and 150% of

Table 2. Intra- and inter-day precision for proposed method

Compound	Amount (ng)	Intra-day ($n = 6$) Amount detected, (mean ± SD)	RSD (%)	Inter-day ($n = 3$) Amount detected, (mean ± SD)	RSD (%)
Citalopram	1000	992.25 ± 5.24	0.52	1008.93 ± 15.25	1.51
	2000	2000.58 ± 12.17	0.60	1981.22 ± 34.36	1.73
	4000	4008.52 ± 35.00	0.87	3992.80 ± 60.88	1.52
Fluoxetine	1000	990.83 ± 18.28	1.84	998.33 ± 24.30	2.43
	2000	1976.67 ± 26.96	1.36	1980.00 ± 40.93	2.07
	4000	4004.17 ± 53.70	1.34	4006.67 ± 67.88	1.69

additional standard solution. As seen in Table 3, the recoveries of citalopram and fluoxetine were found to be in the range of 99.3–100.3% and 99.4–100.4%, respectively. Corresponding RSD values were found to be less than 0.8% for CIT and 1.9% for FLX, thus confirming the accuracy of the method.

Application to Pharmaceutical Formulations

The proposed TLC method was successfully applied to the analysis of citalopram and fluoxetine in pharmaceutical preparations commercially available in Slovakia. Four pharmaceuticals were analyzed ($n = 3$) and the results are summarized in Table 4. The obtained recovery values were found to be 101.10 and 100.50% of the labeled amount for citalopram and 98.75 and 101.85% of labeled amount for fluoxetine, respectively. The low values of relative standard deviation (below 2.2%) for both

Table 3. Accuracy of the developed method

Compound	Excess drug added[a] to the analyte (%)	Amount found[b] (mean ± SD, ng)	Recovery (%)	RSD (%)
Citalopram	0	1002.45 ± 7.90	100.25	0.79
	100	2001.23 ± 16.36	100.15	0.74
	150	2483.45 ± 14.54	99.34	0.58
Fluoxetine	0	996.67 ± 18.93	99.67	1.90
	100	1988.33 ± 33.29	99.42	1.67
	150	2509.75 ± 33.46	100.39	1.33

[a]Matrix containing 1000 ng drug.
[b]Mean value of the three determinations.

Table 4. Application of proposed method to pharmaceutical formulations

Compound	Preparation content, mg	Content found, mg (mean ± SD, $n = 3$)	RSD (%)	Recovery (%)
Citalopram	Citalec, 20	20.22 ± 0.37	1.82	101.10
	Cipralex, 10	10.05 ± 0.15	1.49	100.50
Fluoxetine	Magrilan, 20	19.75 ± 0.43	2.18	98.75
	Floxet, 20	20.37 ± 0.42	2.06	101.85

CIT and FLX indicate the sufficient precision of the method. No interference peaks or matrix effects from the excipients were observed in the chromatograms of the studied pharmaceutical formulations, thus confirming the selectivity of the method.

From these results it can be seen that the developed method is relatively simple, rapid, and reliable for the determination of citalopram and fluoxetine in pharmaceuticals.

Application to Biological Samples

The proposed method was further applied to screening for SSRI antidepresants (CIT, SER, FLX) in real human urine, serum, and blood samples obtained from patients undergoing therapy with citalopram and

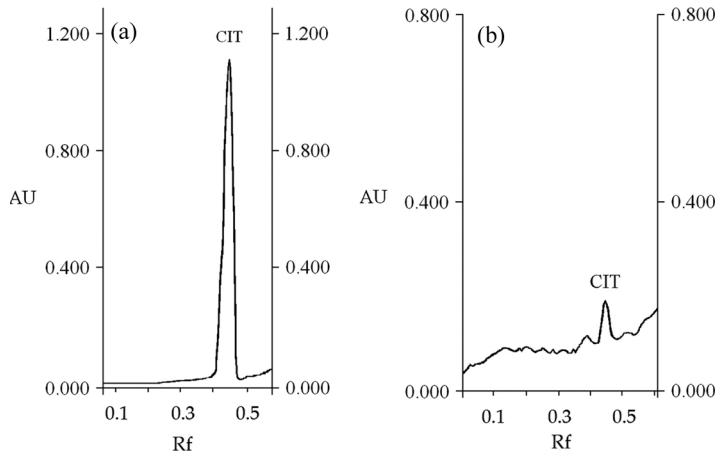

Figure 3. TLC chromatograms of (a) citalopram standard; (b) serum sample from a patient under citalopram treatment (drug dose of 30 mg/day).

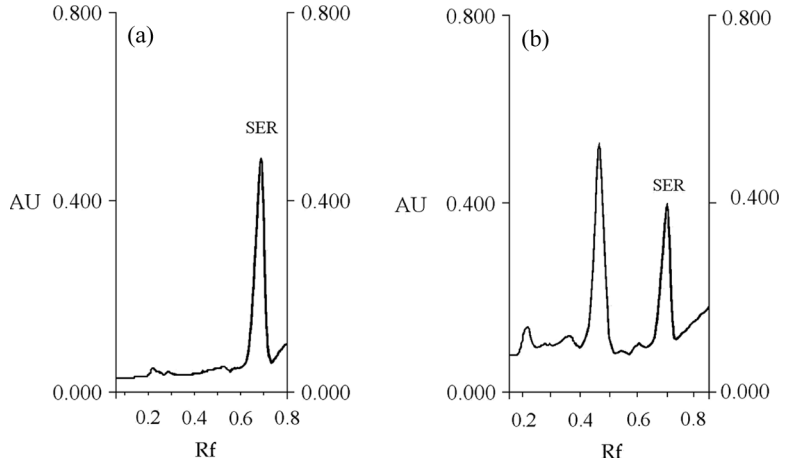

Figure 4. Chromatograms of (a) urine spiked with sertraline; (b) urine sample from a forensic overdose case with sertraline combined with some other drug.

fluoxetine (figure not shown), and from one forensic case after using the corresponding preconcentration procedure described in the Experimental section.

Figure 3 shows chromatograms corresponding to a citalopram standard and a serum sample from a patient under citalopram treatment. Figure 4 illustrates chromatograms of a human urine blank spiked with sertraline (1 μg/mL) and a human urine sample obtained from the forensic case, an individual overdosed with sertraline combined with some other drug. These conclusions were confirmed by the results of the HPLC method.

Under the described chromatographic conditions no interfering peaks of endogenous compounds were observed in the chromatograms of the analyzed biological samples.

CONCLUSION

A simple, rapid, and accurate TLC method using densitometric detection has been developed for simultaneous analysis of four SSRI antidepressants fluoxetine, citalopram, fluvoxamine, and sertraline. The proposed method can be used for the determination of SSRI antidepressants in pharmaceutical preparations. It has the advantages of requiring only simple and reliable instrumentation as well as reducing analysis time and costs.

The developed method with a previous extraction preconcentration step could also be a quite useful screening method for analyzed drugs in overdose or forensic cases.

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REFERENCES

1. Sampson, S.M. Treating depression with selective serotonin reuptake inhibitors. Mayo Clin. Proc. **2001**, *76* (7), 739–744.
2. Mourilhe, P.; Stokes P.E. Risks and benefits of selective serotonin reuptake inhibitors in the treatment of depression. Drugs Safety **1998**, *18* (1), 57–82.
3. Vetulani, J.; Nalepa, I. Antidepressants: past, present and future. Eur. J. Pharmacol. **2000**, *405* (1–3), 351–363.
4. Eap, C.B.; Baumann, P. Analytical methods for the quantitative determination of selective serotonin reuptake inhibitors for therapeutic drug monitoring purposes in patients. J. Chromatogr. B **1996**, *686* (1), 51–63.
5. Lucca, A.; Gentilini, G.; Lopez-Silva, S.; Soldarini, A. Simultaneous determination of human plasma levels of four selective serotonin reuptake inhibitors by high-performance liquid chromatography. Ther. Drug Monit. **2000**, *22* (3), 271–276.
6. Frahnert, C.; Rao, M.L.; Grasmäder, K. Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring. J. Chromatogr. B **2003**, *794* (1), 35–47.
7. Wille, S.M.R.; Maudens, K.E.; van Peteghem, C.H.; Lambert, W.E.E. Development of a solid phase extraction for 13 "new" generation antidepressants and their active metabolites for gas chromatographic-mass spectrometric analysis. J. Chromatogr. A **2005**, *1098* (1–2), 19–29.
8. Berzas, J.J.; Guiberteau, C.; Villasenor, M.J.; Rodriguez, V. Development of a capillary gas chromatographic procedure. Anal. Chim. Acta **2004**, *519* (2), 219–230.
9. Eap, C.B.; Gaillard, N.; Powell, K.; Baumann, P. Simultaneous determination of plasma levels of fluvoxamine and of the enantiomers of fluoxetine and norfluoxetine by gas chromatography mass spectrometry. J. Chromatogr. B **1996**, *682* (2), 265–272.
10. Misztal, G.; Skibinski, R. Chromatographic analysis of new antidepressant drug by normal- and reversed-phase TLC. J. Planar Chromatogr.–Mod. TLC **2001**, *14* (4), 300–304.
11. Berzas, J.J.; Contento, A.M.; Villasenor, M.J.; Aguas, E. Method development and validation for the simultaneous determination of

fluoxetine and fluvoxamine in pharmaceutical preparations by capillary electrophoresis. Anal. Chim. Acta **2000**, *417* (2), 169–176.
12. Schaller, D.; Hilder, E.F.; Haddad, P.R. Separation of antidepressants by capillary electrophoresis with in-line solid-phase extraction using a novel monolithic adsorbent. Anal. Chim. Acta **2006**, *556* (1), 104–111.
13. Sherma, J.; Fried B. Thin-layer chromatographic analysis of biological samples. A review. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28* (15), 2297–2314.
14. Skibinski, R.; Misztal, G.; Kudrzycki, M. Determination of fluoxetine and paroxetine in pharmaceutical formulations by densitometric and videodensitometric TLC. J. Planar Chromatogr.–Mod. TLC **2003**, *16* (1), 19–22.
15. Skibinski, R.; Misztal, G. Determination of fluvoxamine and moclobemide in tablets by densitometric and videodensitometric TLC. J. Planar Chromatogr.–Mod. TLC **2004**, *17* (3), 224–228.

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