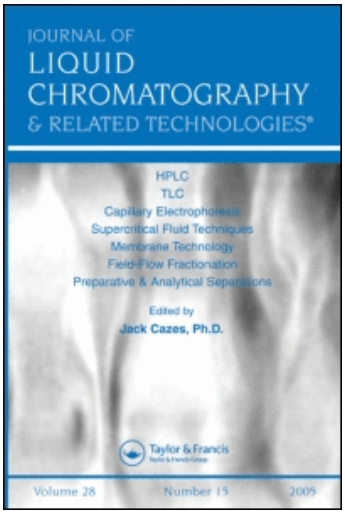

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DETERMINATION OF RESIDUAL PHENOTHIAZINES IN LIVER BY MATRIX SOLID PHASE DISPERSION COMBINED WITH HPLC WITH FLUORESCENCE DETECTION

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□ *A simple and fast method for the determination of phenothiazines in bovine liver has been developed. The analytes were isolated from other liver components by a matrix solid phase dispersion (MSPD) procedure involving a mixture of C_{18} and sea sand as dispersants, a preliminary acidic digestion of the matrix and using acetonitrile as extraction solvent. Because of the complexity of the studied matrix, an extra cleanup of the collected extracts on neutral alumina was required before instrumental determination of the target compounds by high performance liquid chromatography with a fluorescence detector (HPLC-F). The optimised procedure, which involved only 0.250 g of freeze dried sample and a total solvent consumption of 11 mL, allowed accurate determination of the test compounds (recoveries 59–84% and RSD lower than 12% at a spiking level of 200 ng/g wet sample), and limits of detection below 100 ng/g (as calculated for real samples) for all test compounds.*

Keywords fluorescence, liquid chromatography, liver analysis, matrix solid phase dispersion, phenothiazine residues

INTRODUCTION

Phenothiazines are an important group of compounds widely used as therapeutic agents for the treatment of various psychic diseases.[1] Promazines are phenothiazine derivatives belonging to the neuroleptic group. These tranquillizers present an effective antipsychotic effect due to their ability to reduce the activity of dopamine in the central nervous system.[2] Moreover, they are also prescribed as antiemetic and antihistaminic

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medicines.[3] Apart from their use in the treatment of human diseases, these pharmaceuticals are also widely employed in cattle production for either therapeutic reasons or to prevent possible epidemic situations.

In the last years, intensive farming has increased the capacity in meat production at low costs per head. However, this extended, and even abusive, practice causes serious drawbacks, such as increased animal vulnerability to diseases and stress. Stressed animals are susceptible to a high mortality (especially during the transport of cattle to the slaughter house) and often yield poor quality meat products, with the consequence of financial loss. Although the benefits associated to the use of phenothiazines in cattlegrowth promotion are well known,[4] this type of fraudulent practice leads to an accumulation of residues of these drugs in the animal tissues. In the case of food producing animals, such residues can finally affect humans via the food chain.

To protect consumers' health, the European Union (EU) have restricted the usage of a number of these drugs in food producing animals and regulated their maximum residue limits (MRLs) allowed in a variety of meat products.[5] In particular, the use of phenothiazine derivatives (acetylpromazine, chlorpromazine, and propionylpromazine) is banned,[6] although no MRLs have been set for these compounds.[7]

Most of the analytical procedures reported up to now for the determination of promazine derivatives in pharmaceutical preparations,[4] urine,[8,9] blood,[10,11] hair,[12] or liver[13,14] involve a number of steps for exhaustive extraction of the matrices and subsequent cleanup of the extracts before final instrumental determination, which results in laborious and time consuming protocols almost irrespective of the considered matrix.

The feasibility of classical sorbents, such as C_{18}, alumina and silica for preconcentration and cleanup of selected drugs have been demonstrated for both biological fluids[8,15] and previously solubilised tissues.[14–16] For the former sample types, novel polydivinylbenzene based sorbents, e.g., Oasis®, has also been evaluated, alone or in combination with the quoted above classical phases.[6,17,18]

Matrix solid phase dispersion (MSPD) may be an efficient and valid analytical approach when developing faster sample preparation procedures because it allows the extraction and (preliminary) cleanup of the extracts to be carried out in a single step. In most of the MSPD applications, classical sorbents have been used as dispersants for drug analysis in tissues.[16,19] However, to the best of our knowledge, none of these studies led to the determination of promazine derivatives.

The chromatographic separation of phenohtiazines has typically been carried out by high performance liquid chromatography (HPLC)[13–15,20] with a variety of detectors, including UV-vis,[13,14,17,20] chemiluminescent,[9] fluorescent,[14,15] electrochemical,[11] or mass spectrometry (MS).[6,12,15,21]

Nevertheless, some methodologies involving the use of gas chromatography (GC) have also been described.[8,21]

In this study, a MSPD based method previously optimised for the determination of glucocorticoids in liver samples[22] has been evaluated and adapted for the simultaneous enrichment and cleanup of selected promazines in order to allow the analysis of the test compounds by HPLC-F in complex tissue matrices.

EXPERIMENTAL

Reagents

Beef liver samples were purchased from a local butcher's shop, cut in pieces, homogenised, freeze dried and kept at −20°C until analysis.

Pure standards of the target compounds, chlorpromazine hydrochloride, propionylpromazine hydrochloride, and acetylpromazine (Fig. 1), were purchased from Sigma Chemical Co. (St. Louis, USA). Working stock solutions of the individual compounds were prepared in methanol at a concentration level of $1000\,\mu g \cdot mL^{-1}$ and used for further dilution and spiking of the samples. Standard solutions were weekly prepared and conserved at 4°C preserved from light.

All reagents used were of analytical reagent grade. Sodium hydroxide was from Panreac (Barcelona, Spain), hydrochloric acid from Merck (Darmstadt, Germany), and sulphuric acid from Scharlab (Barcelona,

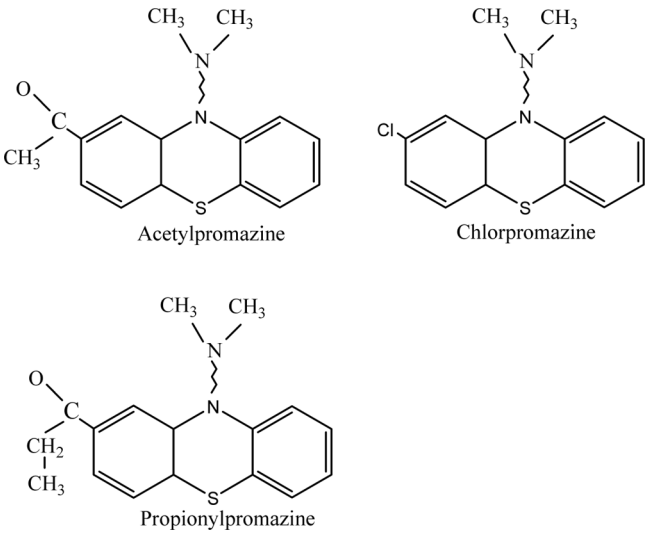

FIGURE 1 Structure of the phenothiazines investigated.

Spain). Ethyl acetate, acetonitrile, and methanol were HPLC grade (Scharlab). Milli-Q water was purified with a Milli Ro Milli Q Plus 185 of Millipore (Waters, Milford, USA).

C_{18} (55–105 μm) was purchased from Waters, active neutral alumina (100–200 mesh) from Bio-Rad (Richmond, CA, USA), and washed sea sand (0.25–0.30 mm) from Panreac.

Apparatus

A Vac Elut system (Varian Ibérica, Madrid, Spain) was used as a vacuum manifold system for sample preparation, and a sample concentrator Techne DRIBLOCK DB 20, equipped with temperature control and N_2 flow (Genesys Instrumentation, Madrid, Spain) were used for concentration of the collected sample extracts.

The HPLC system consisted of a Jasco Analytica (Madrid, Spain) PU-1580 high pressure pumping system equipped with a Rheodyne Model 7125 injector with a 20 μL loop and a Kromasil C-18 column (150 × 4.6 mm; 5 μm; Scharlau). The fluorescence detector was a Jasco Analytica FP-1520. A chromatography interface, "Hercule lite Interface" and the Borwin software (JMBS software for scientists, Jasco Analytica) were used to acquire, analyse, and plot the chromatographic data.

Procedure

In brief, an accurately weighed 0.250 g amount of the freeze dried liver sample (corresponding to 0.500 g of wet sample) was placed in a porcelain mortar and mixed with 1.0 g of C_{18} and 1.0 g of sea sand by gently grounding with a pestle. This treatment allowed the disruption of the sample and its dispersion on the sorbent surface. The homogenous dried mixture was then packed into a glass dispensable extraction cartridge (8.0 × 1.2 cm; J.T. Backer, The Netherlands) equipped with a propylene frit on the bottom and, after adding an upper layer of sea sand, was installed in the vacuum manifold system. After an acidic digestion of the sample with 8 mL of HCl 1 M and a drying step (1 min under vacuum), the sample was extracted with 6 mL of acetonitrile. The eluate was concentrated under a gentle nitrogen current to ca. 500 μL and eluted through a glass dispensable column containing 0.250 g of activated neutral alumina with 5 mL of ethyl acetate:methanol (80:20, v/v).

This purified extract was evaporated to dryness and the residue dissolved in 1.0 mL of the chosen HPLC mobile phase. The resulting solution was filtered through a 0.45 μm disposable syringe filter unit. Of the filtrated solution, 20 μL was injected in the chromatographic system.

Otherwise specified, experiments were carried out in triplicate and each extract injected twice in the HPLC-F system. Experiments were carried out in a fume hood to prevent any possible contamination. Procedural (i.e., reagents) blank and liver blank samples were analysed in each set of experiments to check contamination throughout the analytical method. Blank samples were prepared as for spiked samples but without spiking. No background interference was found to be introduced by the methodology proposed.

RESULTS AND DISCUSSION

Chromatographic Analysis

Some preliminary experiments which consisted of recording both, the excitation and emission spectra of the individual phenothiazines under investigation, were carried out to select the fluorimetric detection conditions. From the spectra recorded, it was observed that the excitation and emission of acetylpromazine and propionylpromazine could be carried out at the same wavelengths: $\lambda_{exc} = 240$ nm and $\lambda_{emi} = 600$ nm. However, chlorpromazine exhibited a different behaviour with a wavelength of maximum fluorescence intensity at $\lambda = 450$ nm when it was excited at 253 nm. Therefore, these two sets of values were selected for the monitoring of the target analytes in the HPLC eluates.

Previously reported studies dealing with the determination of tranquillizers in biological samples have shown the feasibility of C_{18} type phases for this type of HPLC determination.[6,13–15] Therefore, a similar reversed phase type column was chosen in the present study. The chromatographic behaviour of the three test compounds in this stationary phase was evaluated using different methanol:water mixtures, i.e., 80%, 70%, 60%, v/v.

Table 1 summarizes some relevant chromatographic parameters calculated for the target compounds with the several mobile phases

TABLE 1 Retention Time and Chromatographic Resolution Achieved for the Target Compounds Under the Several Experimental Conditions Assayed

	Retention Time (min)		
Analyte	MeOH:H_2O (70:30, v/v)	MeOH:H_2O (70:30, v/v), 1 mM H_2SO_4	MeOH:H_2O (60:40, v/v), 1 mM H_2SO_4
Acetylpromazine	1.675	1.653	2.485
Propionylpromazine	1.985	1.972	3.727
Chlorpromazine	2.522	2.622	4.638
$Rs_{1,2}$	1.0	1.0	3.5
$Rs_{2,3}$	0.7	1.7	2.5

assayed. The flow rate was kept in all instances at 1 mL/min. The mobile phase containing the highest percent of organic modifier, i.e., 80%, resulted in an early elution of the analytes and an insufficient separation among them. A percentage of 70% of methanol in the mobile phase provided enough resolution among the three phenothiazines analysed. However, under these conditions, the baseline did not stabilize after the change in the detection conditions selected for the monitoring of chlorpromazine. The addition of a small percent of sulphuric acid, i.e., 1 mM, led to better defined signals, higher fluorescence intensities, and lower peak widths. Sulphuric acid was selected in order to avoid the possible height atom effect in the detection. Nevertheless, the best chromatographic resolution and fluorescence detection were obtained using a mixture of methanol:water 60:40 (v/v) acidified with 1 mM H_2SO_4. Under these conditions, the fluorescence detector was programmed to change the detection conditions from $\lambda_{exc} = 240$ nm and $\lambda_{emi} = 600$ nm to $\lambda_{exc} = 253$ nm and $\lambda_{emi} = 450$ nm 4.0 min after the starting of the chromatogram.

The analytical performance of the optimised instrumental HPLC-F method was then evaluated. Satisfactory linear calibrations curves were obtained for the three studied phenothiazines with regression coefficients better than $r = 0.996$ (evaluated concentration range 50–1000 ng/mL for acetylpromazine and propionylpromazine and 20–1000 ng/mL for chlorpromazine; six calibration points). The detection limits (signal to noise ratio >3) calculated for acetylpromazine, propionylpromazine, and chlorpromazine were 27, 20, 19 ng/mL, respectively. The minimum determinable amounts (signal to noise ratio >10) were 50 ng/mL for acetylpromazine, 46 ng/mL for propionylpromazine, and 20 ng/mL for chlorpromazine. In addition, the relative standard deviation (RSD) and relative error (Er, %) values were evaluated at three different concentration levels ($n = 5$). In all the cases, RSDs were equal to or less than 13% (corresponding to the value obtained at 100 ng/mL). Meanwhile, the Er values were in the range 3–18% (at 100 ng/mL concentration level).

Sample Preparation

Extraction

The complexity of most of the biological matrices, combined with the extremely low levels at which pharmaceuticals should accurately be detected in them, have made most of the conventional methods used for this type of determination to be laborious and highly manipulative analytical procedures.[13] As previously mentioned, MSPD is considered a valuable alternative when developing simplified analytical procedures for these types of determinations, because this technique allows carrying out

a (preliminary) purification of the extracts at the same time that the extraction step is developed.[16,19] This results in a simplification (or even elimination) of the subsequent cleanup step(-s). Consequently, MSPD was preferred for the liver treatment in the present study.

The MSPD conditions were preliminarily set as those previously optimised for the determination of glucocorticoids in liver because of the satisfactory cleanup achieved in that study.[22] That is, 0.250 g of the lyophilised liver were mixed with 1.0 g of C_{18} and 1.0 g of sand and the homogenous powder packed in a glass dispensable column as described above. As could be anticipated on the basis of the complexity of the investigated matrix, direct elution of the MSPD column with an organic solvent, i.e., either methanol, ethyl acetate, or acetonitrile, resulted in extremely dirty extracts demanding a subsequent laborious and/or highly selective cleanup procedure. Therefore, the possibility of introducing an extra step allowing matrix digestion and (partial) preliminary elimination of this coextracted biogenic material before MSPD column extraction was investigated.

Two types of hydrolysis were assayed for on-column matrix digestion, i.e., acidic, with HCl 1 M, and basic, with NaOH 0,1 M.[21] In both cases, the treatment consisted of two 5 min static extractions and involved a total of 8 mL of the corresponding reagent. Then, the MSPD column was dried for 1 min under vacuum at maximum speed to ensure water removal and the target compounds were extracted with 6 mL of acetonitrile. After a purification step on alumina (see below), the obtained extracts were evaporated to dryness, the residue was reconstituted in 1.0 mL of mobile phase, filtered, and injected in the chromatographic system.

Table 2 summarises the results found in these sets of experiments and illustrates the influence of these hydrolysis treatments on the recoveries and RSDs (n = 3) obtained for the case of a liver spiked at the at the 800 ng/g level (wet basis).

The large number of interferences extracted by direct elution of the MSPD column with an organic solvent was only partially eliminated on the alumina column and consequently hampered the subsequent instrumental determination of the target compounds. Under these conditions, unacceptably low recoveries in the 9–14% range were calculated for the three studied promazines.

TABLE 2 Recoveries and Corresponding RSDs (Between Parenthesis) Calculated for the Target Compounds with the Several Extraction Procedure Assayed (n = 3). Spiking Level, 800 ng/g

Analyte	Without Digestion	NaOH 0.1 M	HCl 1 M
Acetylpromazine	14 (9)	2 (15)	50 (6)
Propionylpromazine	13 (7)	15 (11)	59 (3)
Chlorpromazine	9 (10)	38 (10)	86 (2)

Much cleaner extracts were obtained after alkaline digestion with NaOH 0.1 M, a result that agrees with previous observations from other authors.[21] However, in our case, this treatment apparently caused a large (recoveries for chlorpromazine and propionylpromazine, 38% and 15%, respectively), or even complete degradation for acetylpromazine (recovery, 2%; RSD, 15%) of the target compounds. The most favourable results were obtained after acid digestion with HCl 1 M. With this treatment, recoveries in the 50–86% range, with satisfactory RSDs of 2–6% were obtained for the three tranquillizers investigated. Therefore, this hydrolysis procedure was preferred for subsequent optimisation experiments.

The experiments carried out to optimise the volume of acetonitrile used to elute the target compounds from the MSPD column proved that no further improvement in the analytes recoveries was obtained by increasing this elution volume up to 10 mL. Therefore, a volume of 6 mL was used in subsequent assays.

Cleanup

Despite the significant removal of interferences achieved with the extra digestion step carried out before elution of the studied drugs from the dispersed liver column, subsequent purification of the extracts containing the phenothiazines prior chromatographic determination was still mandatory. On the base of previous results reported for liver,[22] a polar sorbent, i.e., neutral alumina, was selected for selective retention of the polar coextracted compounds remaining in the MSPD eluate.

Experiments were carried out to optimise the selectivity of this purification step. Different solvents, including acetonitrile, ethyl acetate, and mixtures of ethyl acetate:methanol (either 80:20 or 70:30, v/v) were assayed. In all instances, a known amount of the analytes (800 ng/g of each compound, wet basis) was added on top of the alumina column and allowed to stand for 5 min. Then, the studied phenotiazines were eluted with 5 mL of the preselected solvent. Acetonitrile was first tested as the elution solvent, because the use of this solvent would allow a direct coupling between the MSPD column and this purification cartridge. Unfortunately, acetonitrile resulted in an uncompleted elution of the target compounds from the alumina column regardless of the sorbent:solvent ratio used (recoveries in the 5–15% range) and, consequently, was not further considered as the extraction solvent for this purification step.

Table 3 summarises the results obtained when using ethyl acetate and mixtures of this solvent with methanol (i.e., with 20% or 30% of methanol) as extraction solvent. The best results were obtained with the ethyl acetate: methanol 80:20 (v/v), which provided satisfactory recoveries for the three analytes (in the range 85–95%). Higher methanol percentages reduced

TABLE 3 Influence of the Extraction Solvent Nature on the Recoveries of the Analytes from an Alumina Column (n = 3). Spiking Level, 800 ng/g

Analyte	Ethyl Acetate	Ethyl Acetate:MeOH (80:20, v/v)	Ethyl Acetate:MeOH (70:30, v/v)
Acetylpromazine	36	91	164
Propionylpromazine	85	95	94
Chlorpromazine	69	85	109

significantly the selectivity of the purification process. Meanwhile, the use of pure ethyl acetate reduced the efficiency of the extraction and entailed lower recoveries values for all the compounds (in the 36–85% range).

Method Evaluation

Calibration graphs from unfortified (i.e., non-spiked liver) and fortified samples at concentration levels ranging from 100 to 800 ng/g (wet basis) were constructed. Excellent linear relations were obtained for all the analytes studied, as it is shown by the satisfactory correlation coefficients ($r > 0.998$). The sensitivity for each compound is given by the calibration graph slope values shown in Table 4, corresponding to 0.128, 0.159, and 1.738 area × g/ng for acetylpromazine, propionylpromazine, and chlorpromazine, respectively. The LODs and LOQs for the target compounds were calculated from unfortified samples as described above. As shown in Table 4, the minimum detectable concentrations were in all instances below 100 ng/g, a value that is in the range of those previously reported for the determination of these drugs in liver, but using much larger amounts of sample (i.e., 5 g) and more laborious treatment protocols.[14]

The recovery, accuracy, and precision of the complete method were evaluated at three concentration levels, 200, 400, and 800 ng/g (wet basis), for the three promazine derivatives. At spiking values similar to or above 400 ng/g, recoveries consistently better than 83% and RSD below 6% (n = 3) were obtained. These results agree with those previously reported in the literature for similar applications but involving larger amounts of sample, more tedious sample preparation protocols, and a much higher spiking level of 10–20 μg/g.[14] Although, somehow lower recoveries were found at the lowest spiking level assayed of 200 ng/g (in the 59–84% range), the still acceptable reproducibility (RSD values below 12%) could made the method to be considered as suitable even at such low concentration levels. More importantly, these values and the low Er (lower than 25% in all instances) proved that the proposed method can be applied with enough accuracy and precision to the analysis of very complex matrices, such as bovine liver, at the low levels investigated, even if a relatively simple

TABLE 4 Relevant Analytical Data Related to the Developed Analytical Method (n = 3)

Analyte	Slope (area × g/ng)	LOD (ng/g)	LOQ (ng/g)	Er (%)			RSD			Recovery (%)		
				200 (ng/g)	400 (ng/g)	800 (ng/g)	200 (ng/g)	400 (ng/g)	800 (ng/g)	200 (ng/g)	400 (ng/g)	800 (ng/g)
Acetylpromazine	0.128	100	140	≤25	≤22	≤19	12	6	2	59	83	86
Propionylpromazine	0.159	90	135	≤19	≤14	≤11	11	5	2	67	83	95
Chlorpromazine	1.738	80	110	≤12	≤13	≤10	9	4	3	84	92	92

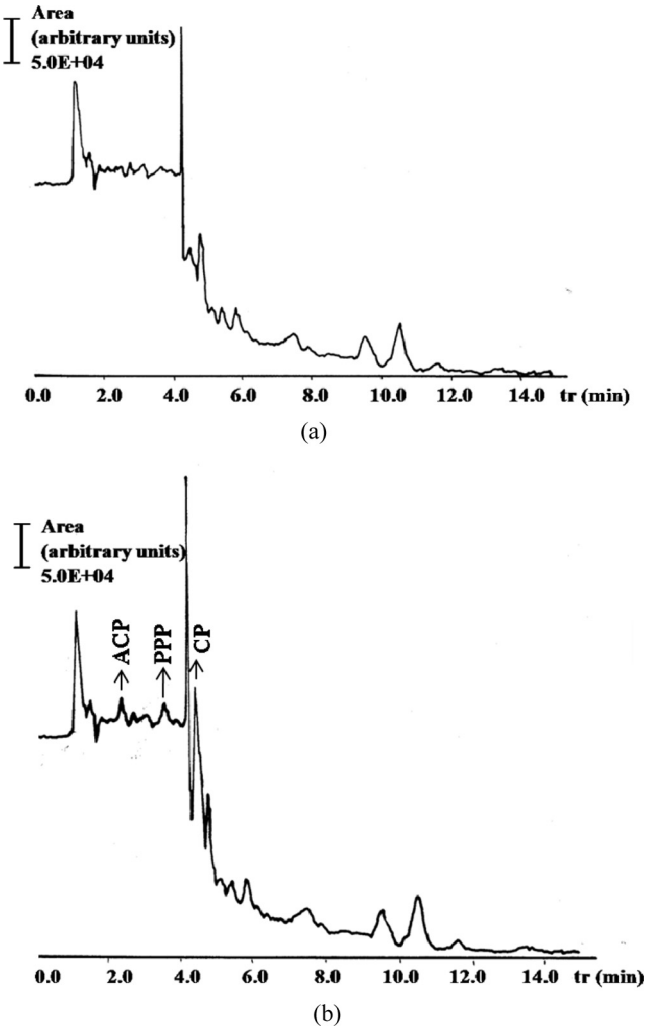

FIGURE 2 Typical HPLC-F chromatograms of the liver extracts obtained using the proposed method for (a) a non-spiked sample and (b) a spiked sample (spiking level, 400 ng/g, wet basis).

HPLC-F system was used for final determination of the analytes. The clean chromatograms obtained (Fig. 2) despite the relative simplicity of the sample preparation procedure used, contribute to supporting this statement.

CONCLUSIONS

A relatively simple sample preparation method for the analysis of selected phenothiazines in bovine liver has been proposed and evaluated.

The developed procedure allowed fast treatment of this complex matrix with minimum solvent consumption (11 mL per sample) and provided sensitivities and reproducibilities in the order of those previously reported for more laborious and manipulative large scale procedures. These features made the proposed procedure to be considered as a valuable analytical alternative for the routine residue monitoring of promazine derivatives in complex tissue samples, including liver.

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REFERENCES

1. Dahl, S.G. *Clinical Pharmacology and Psychiatry, Neuroleptic Antidepressants Research*; (Second International Meeting 1980), Usdin, E., Dahl, S.G., Gram, L.F., Eds.; Macmillan: London, 1981, 125–137.
2. Jiang, M.X.; Yang, Z.H. *Pharmacology*, 4th ed.; Chinese Hygienic Press: Beijing, 1996.
3. Martindale, *The Complete Drug Reference*; Parfitt, K., Pharmaceutical Press: London, **1999**, *63*, 5–700.
4. Pérez-Ruiz, T.; Martínez-Lozano, C.; Sanz, A.; San Miguel, M.T. Flow inyection chemiluminiscence determination of phenothiazines in pharmaceutical preparations. Lab. Autom. Inf. Manag. **1999**, *34* (2), 149–158.
5. EEC Council Directive 96/23/ECC, Offic. J. Eur. Commun., L125, **1996**, 10.
6. Delahaut, P.; Levaux, C.; Eloy, P.; Dubois, M. Validation of a method for detecting and quantifying tranquillisers and a β-blocker in pig tissues by liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta **2003**, *483*, 335–340.
7. Council Decision amending EEC Council Regulation 90/2377, Offic. J. Eur. Commun. L17, **1997**, 12.
8. Olmos-Carmona, M.L.; Hernández-Carrasquilla, M. Gas chromatographic-mass spectrometric analysis of veterinary tranquillizers in urine: evaluation of method performance. J. Chromatogr. B **1999**, *734* (1), 113–120.
9. Shi, W.; Yang, J.; Huang, Y. Ion – pair complex-based solvent extraction combined with chemiluminescence determination of chlorpromazine hydrochloride with luminal in reverse micelles. J. Pharm. Biomed. Anal. **2004**, *36*, 197–203.
10. Madej, K.; Wozniakiewicz, M.; Kala, M. Method for screening and quantification of seven phenothiazines in whole blood samples by non aqueous capillary electrophoresis. Chromatographia. **2005**, *61* (5/6), 559–263.
11. Ni, Y.; Wanga, L.; Kokot, S. Voltammetric determination of chlorpromazine hydrochloride and promethazine hydrochloride with the use of multivariate calibration. Anal. Chim. Acta **2001**, *439*, 159–168.
12. McClean, S.; O'Kane, E.J.; Smyth, W.F. Electrospray ionization-mass spectrometric characterization of selected antipsychotic drugs and their detection and determination in human hair samples by liquid chromatography tandem mass spectrometry. J. Chromatogr. B **2000**, *740* (2), 141–157.
13. Quintana, M.C.; Blanco, M.H.; Lacal, J.; Hernández, L. Analysis of pharmaceutical residues in bovine liver by HPLC. J. Liquid Chromatogr. & Rel. Technol. **2001**, *24* (5), 735–745.
14. Quintana, M.C.; Blanco, M.H.; Lacal, J.; Hernández, L. Analysis of promazines in bovine livers by high performance liquid chromatography with ultraviolet and fluorimetric detection. Talanta **2003**, *59*, 417–422.
15. Hayen, H.; Karst, U. Analysis of phenothiazine and its derivatives using LC/electrochemistry/MS and LC/electrochemistry/fluorescence. Anal. Chem. **2003**, *75*, 4833–4840.

16. Kristenson, E.M.; Ramos, L.; Brinkman, U.A.T. Recent avances in matrix solid-phase dispersion. TRAC **2006**, *25*, 96–112.
17. Barker, S.A. Applications of matrix solid-phase dispersion in food analysis. J. Chromatogr. A. **2000**, *880* (1 + 2), 63–68.
18. Quintana, M.C.; Ramos, L.; González, M.J.; Blanco, M.H.; Hernández, L. Development of a solid phase extraction method for simultaneous determination of corticoids and tranquilizers in serum samples. J. Sep. Sci. **2004**, *27*, 53–58.
19. Lara, F.J.; García-Campaña, A.M.; Alés-Barrero, F.; Bosque-Sendra, J.M. Development and validation of a capillary electrophoresis method for the determination of phenothiazines in human urine in the low nanogram per millilitre concentration range using field-amplified sample inyection. Electrophoresis **2005**, *26*, 2418–2429.
20. Madej, K.; Kala, M.; Wózniakiewicz, M. LC and non aqueous CE determination of phenothiazines in autopsy samples. Chromatographia **2005**, *62* (9/10), 533–538.
21. Shen, M.; Xiang, P.; Wu, H.; Shen, B.; Huang, Z. Detection of antidepressant and antipsychotic drug in human hair. Foren. Sci. Int. **2002**, *126* (2), 153–161.
22. Ramos, J.J.; González, M.J.; Ramos, L. New Solid Phase Dispersion-based method combined with molecular imprinted polymer purification for the determination of selected glucocorticoids in liver. (in preparation).