https://doi.org/10.5272/jimab.2020264.3403

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2020 Oct-Dec;26(4)

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Original article

IDENTIFICATION AND QUANTIFICATION OF VERAPAMIL IN BLOOD AND URINE

Georgi Bonchev[1], Snezha Zlateva[1,2], Petko Marinov[1,2], Ivelina Stefanova[1]
1) Clinic for Intensive Treatment of Acute Intoxications and Toxicoallergies, Naval Hospital – Varna, Military Medical Academy, Bulgaria
2) Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University - Varna, Bulgaria.

ABSTRACT

Purpose: To adapt and validate an HPLC method for verapamil determination in blood and urine samples.

Materials/Methods: Identification of verapamil and its metabolites was made by means of gas-chromatography, using Agilent 7890B/5977A GC-MS system featuring a DB-1701 column. Quantification was done by means of liquid chromatography on Agilent 1260 series HPLC, equipped with Zorbax Extend-C18 column and both diode-array and fluorescent detection modules. Blood and urine specimens were taken from patients of the Clinic for intensive treatment of acute intoxications and toxicoallergies within the course of their treatment.

Results: GC-MS identification of verapamil and its metabolites was carried out after simple liquid-liquid extraction of samples without further chemical derivatization. Adapted HPLC method for quantification require isocratic conditions and mobile phase, consisted of phosphate buffer (pH 2.7; 10 mM) containing 1.5 ml L^{-1} triethylamine – acetonitrile (70:30, v/v) at 20°C, flow-rate 1.0 mL/min and FLD detection (excitation: 203 nm, emission: 320 nm). The method was demonstrated to be linear within the whole region of interest (4.6-4600 ng mL^{-1}) with excellent accuracy (101.7-102.2%) and inter-day precision (5.81%) as well as good analytical recovery (81.2%) and LOQ (7.0 ng mL^{-1}).

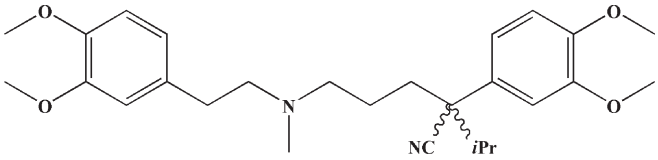
Conclusion: A precise and easy to use method for verapamil detection and quantification is developed. The method is applicable as a routine procedure in the Laboratory of analytical toxicology for both diagnosis clarification in cases of acute intoxications and therapeutic drug monitoring.

Keywords: verapamil, GC-MS, HPLC, acute intoxications, therapeutic drug monitoring

INTRODUCTION

Verapamil (Fig. 1) is an antihypertensive and antiarrhythmic medication of calcium channel blocker family, used for the treatment of HBP [1, 2], angina pectoris [3] and supraventricular tachycardia [4], given orally or *i.v.* It is also known to possess anti-manic properties [5, 6], and although not as effective as valproate or lithium, for example, it could be the medication of choice in specific occasions due to its very low teratogenic profile [7].

Fig. 1. Chemical structure of verapamil. The applicable form is usually hydrochloride.



Common dosage corresponds to blood verapamil therapeutic concentration of 20-250 ng mL^{-1}, acute intoxications lead to values close to 1000 ng mL^{-1}, and levels above 2500 ng mL^{-1} are considered comatose-fatal [8]. Side effects are reportedly common (11.3%) and include constipation (4.03%), dizziness (3.65%), headache (1.54%), and other (less than 1%); as a result in approximately 3% of cases, therapy may require premature discontinuation [9].

Taken in quantities above the toxic dose, verapamil may cause any of following principal effects: hypotension due to arterial vasodilatation, bradycardia and atrioventricular block and cardiogenic shock secondary to a negative inotropic effect; hyperkalemia and metabolic acidosis are also possible [10-16]. Recently, intravenous lipid emulsion (ILE) infusion therapy is repeatedly reported to be highly effective, especially in cases of massive overdoses [17-21].

After oral ingestion, verapamil is effectively absorbed (above 90%), but bioavailability is low (10-35%) due to its extensive first-pass metabolism; plasma protein binding is estimated to be 90% [22]. It undergoes a liver degradation involving CYP450 isoforms, mainly by oxidative dealkylation to more than 30 identified metabolites, notably partially active (approx. 20%) norverapamil (*N*-desmethylverapamil) [23-25]. Excretion of verapamil is by the kidney with an elimination half-life of 2.8-7.4 hours; only 3-4% of the initial drug is in unchanged form and roughly 70% as metabolites [22].

Although acute intoxications with calcium channel blockers in general and with verapamil, in particular, are rare, the mortality rate may exceed 10% [26]. That is why any laboratory of analytical toxicology should maintain appropriate techniques for verapamil determination. For identification purposes, GC-MS is considered a gold standard because of its versatility of toxicological applications as well as unambiguity of its results [27-28]. Quantification could also be done by gas chromatography, although liquid techniques, such as HPLC (preferably in tandem with fluorescence detection) are more convenient [25, 29-33]. Sample preparation routinely includes liquid-liquid extraction, and however, solid-phase extraction methods have also been proposed [34]. Critically studying the literature available, one can notice that developed methods for verapamil quantification are mostly optimized for precision and sensitivity and, therefore, require either complicated hyphenated equipment, arduous multi-step procedures and/or complex extragent/eluent mixtures. As clinical work at toxicology units is often urgent, rapidity of results and simplicity of lab work may be of greater importance. Therefore a simple, yet effective procedure should be developed by optimization of the available techniques.

MATERIALS AND METHODS

Analytical identification of organic compounds relied on Agilent 7890B/5977A GC-MS system equipped with a DB-1701 column (30 m × 0.250 mm × 0.25 μm). The HPLC analysis was done onto Agilent 1260 Infinity system featuring Zorbax Extend-C18 column (150 mm × 4.6 mm × 5 μm) and DAD/FLD detection modules. Human blood and urine samples originated from controlled stationary patients of Naval Hospital – Varna. Deionized water (0.067-0.100 μS cm–1, TKA™ Pacific water purification system), HPLC grade solvents, and only analytical grade chemicals were used. Agilent OpenLAB (ChemStation edition, rev. C.01.05), MassHunter (rev. B.07.00), and spectral library NIST (ver. 2.0) software were used for chromatographic data acquisition and manipulation. Statistical processing was done by MS Excel™ and OriginPro® software.

RESULTS AND DISCUSSION

For paraclinical identification of verapamil intoxication, a 4 mL urine sample is required. The sample is processed by routine GC-MS screening procedure, including initial deproteinization (500 μL acetonitrile), followed by simple liquid-liquid extraction (4 mL ethyl acetate in alkaline conditions); organic (upper) layer is transferred, dried (100 mg anhydrous $MgSO_4$), and centrifuged (2 min at 4000 rpm). The solvent is evaporated to dryness (60°C under N_2 stream), and the residue is reconstituted in 50 μL of methanol. The GC-MS injection volume is 1 μL; operation conditions are given in Tabl. 1. Verapamil peak is always present in chromatograms of positive cases at R_t = 42.4 min, mass spectrum (EI, 70 eV): *m/z* (I_{rel}, %): 303 (100), 304 (23), 58 (17), 151 (12). Depending on specific conditions (e.g. the extent of intoxication, time from the event and the measures taken), series of metabolites could be identified as well, most notably norverapamil at R_t = 43.6 min, mass spectrum (EI, 70 eV): *m/z* (I_{rel}, %): 289 (100), 290 (21), 151 (19), 152 (12) and N-desalkylverapamil at R_t = 24.7 min, mass spectrum (EI, 70 eV): *m/z* (I_{rel}, %): 57 (100), 164 (78), 290 (73), 247 (55), 70 (49).

Tabl. 1. GC-MS Operation conditions, SCAN mode.

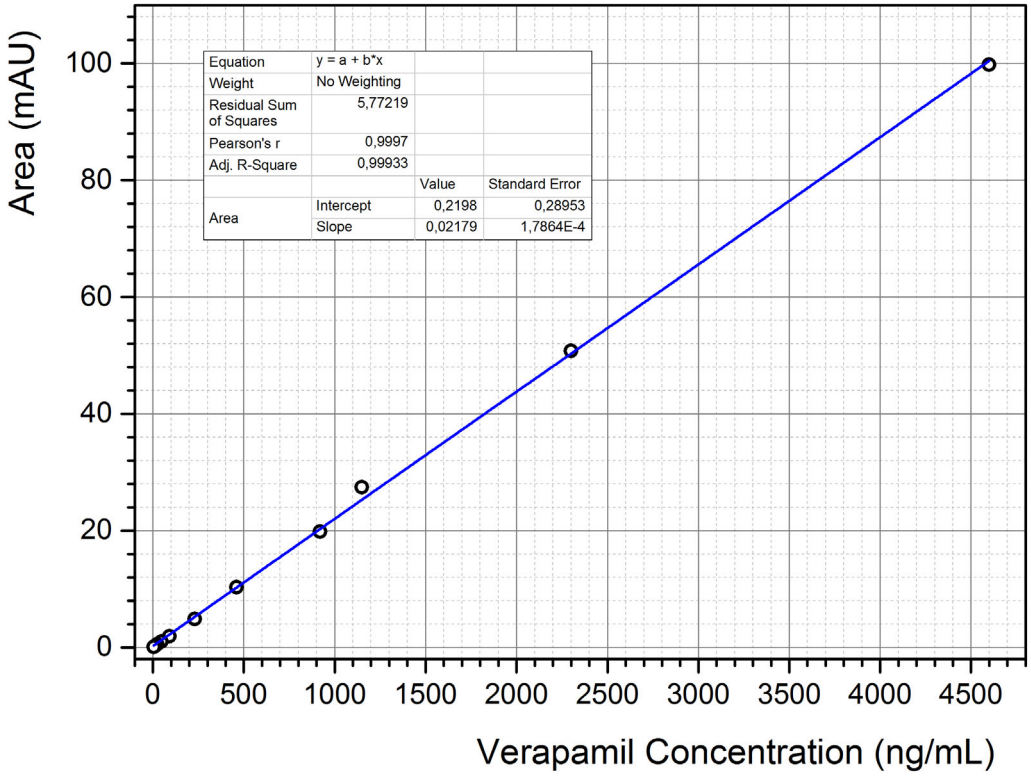
Parameter	Value	Parameter	Value
Initial oven temp.	50°C	GC Column	DB-1701
Initial time	2 min	Column dimensions	30 m × 0.250 mm
Oven ramp rate	20°C min–1	Film thickness	0.25 ìm
Oven final first ramp	90°C	Inlet mode	splitless
Final time first ramp	1 min	Flow mode	constant flow
Oven ramp rate	8°C min–1	Flow rate	1.5 mL min–1
Oven final temp.	280°C	Carrier gas	Hellium (He)
Final time	15 min	Ion source temp.	230°C
Total run time	43.75 min	Inlet temp.	250°C

Analytical quantification of verapamil is made by HPLC, applying external calibration approach; 0.5 mL blood serum/plasma sample is required. Sample preparation goes trough alkalinization (500 μL 1 M NaOH), deproteinization (1.5 mL acetonitrile) and double liquid-liquid extraction (2 × 3 mL of ethyl acetate), desiccation

of combined extracts (100 mg anhydrous $MgSO_4$), centrifugation (1 min at 4000 rpm), and evaporation (60°C under N_2 stream). The residue is reconstituted in 500 µL of the mobile phase followed by syringe filtration (0.22 ìm, Nylon); 20 µL of filtrate are injected on the HPLC column. Liquid chromatography was done under isocratic conditions. Mobile phase consisted of phosphate buffer (pH 2.7; 10 mM) containing 1.5 ml L^{-1} triethylamine – acetonitrile (70:30, v/v) at 20ºC, flow-rate 1.0 mL/min and FLD detection (excitation: 203 nm, emission: 320 nm). We tried to use a UV-DAD detection (λ=222, 224, 226, 278, 298 nm) only to found this approach inadequate, as even at the highest concentration used the quality of the signal was not satisfactory. Retention times were between 3.9 and 4.1 min.

Validation of the method follows the recommendations of the Internationaal Committee of Harmonization (ICH) as well as the United Nations Office on Drug and Crimes (UNODC) protocols for analysis of biological specimens. Stock verapamil solution (4.6 µg mL^{-1}) was prepared by diluting 1 mL of the original substance (2.5 mg mL^{-1} verapamil.HCl) with a mobile phase. Ten standard solutions (4.6-4600 ng mL^{-1}) were prepared by progressive dilutions, analyzed, and results fitted linearly (Fig. 2). The model was linear over the whole concentration range (4.6-4600 ng mL^{-1}) with Pearson's R^2=0,9994. Inter-day precision was estimated to 0.55% at 920 ng mL^{-1} and 5.8% at 11.5 ng mL^{-1}. Intra-day precision (one week) at 920 ng mL^{-1} equals 13.8%. Accuracy was determined for low (101.7% at 23 ng mL^{-1}) and high (102.2% at 920 ng mL^{-1}) concentration zones. Spiking blank blood samples, 81.2% analytical recovery was determined. Limits of detection (LOD) and quantification (LOQ) are estimated to be 2.1 ng mL^{-1} and 7.0 ng mL^{-1}, respectively. Stability of retention time was within 0.1 min over 10 days period. A single experimental cycle takes approximately 45 minutes altogether, including sample preparation, HPLC analysis and administrative information maintenance.

Fig. 2. Linear regression fit of standard solutions for HPLC calibration.



CONCLUSION

Rapid, stable and precise HPLC method for the quantitative determination of verapamil was developed, optimized and applied in clinical practice. The method is a useful asset for the Lab of Analytical toxicology as it helps in diagnostics and treatment processes. Identification of acute intoxications is done my GC-MS and targets verapamil itself as well as some of its metabolites.

REFERENCES:

1. Lewis GR, Morley KD, Lewis BM, Bones PJ. The treatment of hypertension with verapamil. *N Z Med J*. 1978 May 24;87(612):351-4. [PubMed]

2. Anavekar SN, Christophidis N, Louis WJ, Doyle AE. Verapamil in the treatment of hypertension. *J Cardiovasc Pharmacol*. 1981 Mar-Apr;3(2):287-92. [PubMed]

3. Frishman WH, Charlap S. Verapamil in treatment of chronic stable angina. *Arch Intern Med*. 1983 Jul;143(7):1407-15. [PubMed]

4. Krikler DM, Spurrell RA. Verapamil in the treatment of paroxysmal supraventricular tachycardia. *Postgrad Med J*. 1974 Jul;50(585):447-53. [PubMed]

5. Giannini AJ, Houser WL Jr, Loiselle RH, Giannini MC, Price WA. Antimanic effects of verapamil. *Am J Psychiatry*. 1984 Dec;141(12):1602-3. [PubMed]

6. Giannini AJ, Taraszewski R, Loiselle RH. Verapamil and lithium in maintenance therapy of manic patients. *J Clin Pharmacol*. 1987 Dec;27(12):980-2. [PubMed]

7. Alabdulrazzaq F, Koren G. Fetal safety of calcium channel blockers. *Can Fam Physician*. 2012 Jul;58(7):746-7. [PubMed]

8. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Crit Care*. 2012 Jul 26;16(4):R136. [PubMed]

9. Speders S, Sosna J, Schumacher A, Pfennigsdorf G. Efficacy and safety of verapamil SR 240 mg in essential hypertension: results of a multicentric phase IV study. *J Cardiovasc Pharmacol*. 1989;13 Suppl 4:S47-9. [PubMed]

10. Immonen P, Linkola A, Waris E. Three cases of severe verapamil poisoning. *Int J Cardiol*. 1981;1(1):101-5. [PubMed]

11. Pritza DR, Bierman MH, Hammeke MD. Acute toxic effects of sustained-release verapamil in chronic renal failure. *Arch Intern Med*. 1991 Oct;151(10):2081-4. [PubMed]

12. Ashraf M, Chaudhary K, Nelson J, Thompson W. Massive overdose of sustained-release verapamil: a case report and review of literature. *Am J Med Sci*. 1995 Dec;310(6):258-63. [PubMed]

13. Klimaszyk D. [ECG disorders in the course of acute suicidal poisonings by verapamil]. [in Polish] *Przegl Lek*. 2000; 57(10):600-5. [PubMed]

14. Nickson CP, Little M. Early use of high-dose insulin euglycaemic therapy for verapamil toxicity. *Med J Aust*. 2009 Sep 21;191(6):350-2. [PubMed]

15. Osthoff M, Bernsmeier C, Marsch SC, Hunziker PR. Levosimendan as treatment option in severe verapamil intoxication: a case report and review of the literature. *Case Rep Med*. 2010; 2010.pii:546904. [PubMed]

16. Izdes S, Altintas ND, Soykut C. Acute respiratory distress syndrome after verapamil intoxication: case report and literature review. *Acta Clin Belg*. 2014 Apr;69(2):116-9. [PubMed]

17. Liang CW, Diamond SJ, Hagg DS. Lipid rescue of massive verapamil overdose: a case report. *J Med Case Rep*. 2011 Aug 20;5:399. [PubMed]

18. Sampson CS, Bedy SM. Lipid emulsion therapy given intraosseously in massive verapamil overdose. *Am J Emerg Med*. 2015 Dec;33(12):1844.e1. [PubMed]

19. Mandigers L, Bollen PD, Bijlstra PJ, Brands E. Severe verapamil intoxication despite correct use of low-dose verapamil. *Drug Metab Pers Ther*. 2016 Mar;31(1):55-8. [PubMed]

20. Tulgar S, Kose HC, Demir Piroglu I, Karakilic E, Ates NG, Demir A, et al. Comparison of effects of separate and combined sugammadex and lipid emulsion administration on hemodynamic parameters and survival in a rat model of verapamil toxicity. *Med Sci Monit*. 2016 Mar 25;22:984-90. [PubMed]

21. Akgün Þahin F, Çelebi SH, Güngör Ý, Coþkun D, Ergüven Kaya E. Therapeutic effects of intralipid and medialipid emulsionsin a rat model of verapamil toxicity. *Turk J Med Sci*. 2016 Nov 17;46(5):1568-1572. [PubMed]

22. DRUGBANK: Verapamil. [Retrieved 2019 Jul 10] [Internet]

23. Tracy TS, Korzekwa KR, Gonzalez FJ, Wainer IW. Cytochrome P450 isoforms involved in metabolism of the enantiomers of verapamil and norverapamil. *Br J Clin Pharmacol*. 1999 May;47(5):545-52. [PubMed]

24. Borlak J, Walles M, Levsen K, Thum T. Verapamil: metabolism in cultures of primary human coronary arterial endothelial cells. *Drug Metab Dispos*. 2003 Jul;31(7):888-91. [PubMed]

25. Walles M, Thum T, Levsen K, Borlak J. Metabolism of verapamil: 24 new phase I and phase II metabolites identified in cell cultures of rat hepatocytes by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2003 Dec 25;798(2):265-74. [PubMed]

26. Charpentier C, Flandrois M, Labombarda F, Maragnes P, Jokic M, Villedieu F. [Verapamil intoxication: beware of the delayed effect]. [in French] *Arch Pediatr*. 2014 Dec; 21(12):1344-7. [PubMed]

27. Thomson BM, Pannell LK. The analysis of verapamil in postmortem specimens by HPLC and GC. *J Anal Toxicol*. 1981 May-Jun;5(3): 105-9. [PubMed]

28. Bhatia NM, Pathade PA, More HN, Choudhari PB, Jadhav SD, Bhatia MS, et al. Synthesis and characterization of norverapamil and quantification of verapamil and nor-verapamil in plasma. *J Anal Chem*. 2013 Oct;68(10):924-30. [Crossref]

29. Hynning PA, Anderson P, Bondesson U, Boreus LO. Liquid-chromatographic quantification compared with gas-chromatographic-mass-spectrometric determination of verapamil and norverapamil in plasma. *Clin Chem*. 1988 Dec;34(12): 2502-3. [PubMed]

30. Ellenhorn MJ. Ellenhorn's Medical Toxicology: Diagnosis and Treatment of Human Poisoning. Schonwald SN, Ordog G, Wasserberger J. (Eds.) Williams & Wilkins. Subsequent edition. January

1, 1997. 534 p.

31. Sawicki W. A validated method for the determination of verapamil and norverapamil in human plasma. *J Pharm Biomed Anal.* 2001 Jun;25(3-4):689-95. [PubMed]

32. Chytil L, Strauch B, Cvacka J, Maresova V, Widimsky J Jr, Holaj R, et al. Determination of doxazosin and verapamil in human serum by fast LC-MS/MS: application to document non-compliance of patients. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2010 Nov 15;878(30):3167-73. [PubMed]

33. Yazan Y, Bozan B. Rapid analysis of verapamil in plasma by reversed phase HPLC. *Pharmazie.* 1995 Feb;50(2):117-9. [PubMed]

34. Ivanova V, Zendelovska D, Stefova M, Stafilov T. HPLC method for determination of verapamil in human plasma after solid-phase extraction. *J Biochem Biophys Methods.* 2008 Apr 24;70(6):1297-303. [PubMed]

Please cite this article as: Bonchev G, Zlateva S, Marinov P, Stefanova I. Identification and quantification of verapamil in blood and urine. *J of IMAB.* 2020 Oct-Dec;26(4):3403-3407. DOI: https://doi.org/10.5272/jimab.2020264.3403

Received: 16/07/2019; Published online: 29/10/2020

Address for correspondence:
Georgi Bonchev, PhD
Head, Laboratory of Analytical Toxicology, Military Medical Academy, Naval Hospital – Varna
3, Chr. Smirnenski St, Varna – 9010, Bulgaria
E-mail: toxilab.varna@abv.bg,