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# High-performance liquid chromatographic method for the assay of verapamil hydrochloride and related compounds in raw material*

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**Abstract**: A modification of the USP HPLC method [*United States Pharmacopeia XXII*, pp. 1444–1446] for the assay of the purity of verapamil hydrochloride has been evaluated for the determination of the drug content and related compounds in drug raw material. The method enables the resolution of 16 related compounds from the parent drug and, in most cases, from each other. The minimum quantifiable amount for most related compounds is less than 0.05%. Six drug raw material samples are analysed and the total impurities found to be 0.3% or less. All drug assay values were within the USP recommended limits of 99.0–100.5%.

**Keywords**: *Verapamil; related compounds; HPLC assay; impurity profile.*

## Introduction

The authors' laboratory maintains an ongoing programme to ensure the availability of selective and sensitive methods for the determination of impurities in drug raw materials. When available, compendial methods are evaluated and, if found suitable, used for this purpose. Otherwise, existing methods are modified or new methods are developed.

Verapamil hydrochloride is an anti-anginal, anti-arrhythmic and antihypertensive drug. The USP monographs for verapamil hydrochloride raw material, injection and tablets [1] provide an HPLC method for determination of the drug and selected related compounds. Whilst the method enables the resolution of all available related compounds from the drug (Table 1 and Fig. 1), several coelute near the solvent front, and one **XV** requires over 200 min for elution. The BP TLC methods [2] do not resolve **XI** from the drug, nor are all other available related compounds resolved from each other. Unresolved compounds cannot be determined individually to test for compliance with the specifications set in the monograph.

Several methods for the asay of verapamil hydrochloride in dosage forms have been reported [3–5]. HPLC with fluorescence detection [6, 7] and capillary GC with nitrogen–phosphorus detection [8, 9] have been used for the determination of verapamil in biological fluids. None of these methods provided data on the resolution of the available related compounds from the drug.

## Experimental

### *Chemicals*

Acetonitrile (Baker, Phillipsburg, NJ, USA), glacial acetic acid (Caledon Laboratories, Georgetown, Ontario, Canada), sodium acetate (Fisher Scientific, Fairlawn, NJ, USA) and 2-aminoheptane (Aldrich, Milwaukee, WI, USA) were HPLC grade. Deionized water was used. Verapamil related compounds used were obtained as follows: USP Reference Standard Verapamil Related Compounds A and B from USPC, Rockville, MD, USA; **I**, **IV**, **VII** and **VIII** from Aldrich Chemical Co., Milwaukee, WI, USA; **II**, **VI**, **IX**, **X**, **XI**, **XII**, **XIII**, **XIV** and **XV** from Knoll Pharmaceuticals, Ludwigshafen, Germany; **III** and **XVI** from Orion Corp, Espoo, Finland; **V** from Secifarma, Milan, Italy. The mass, IR and proton magnetic resonance spectra of these compounds were consistent with their respective structures.

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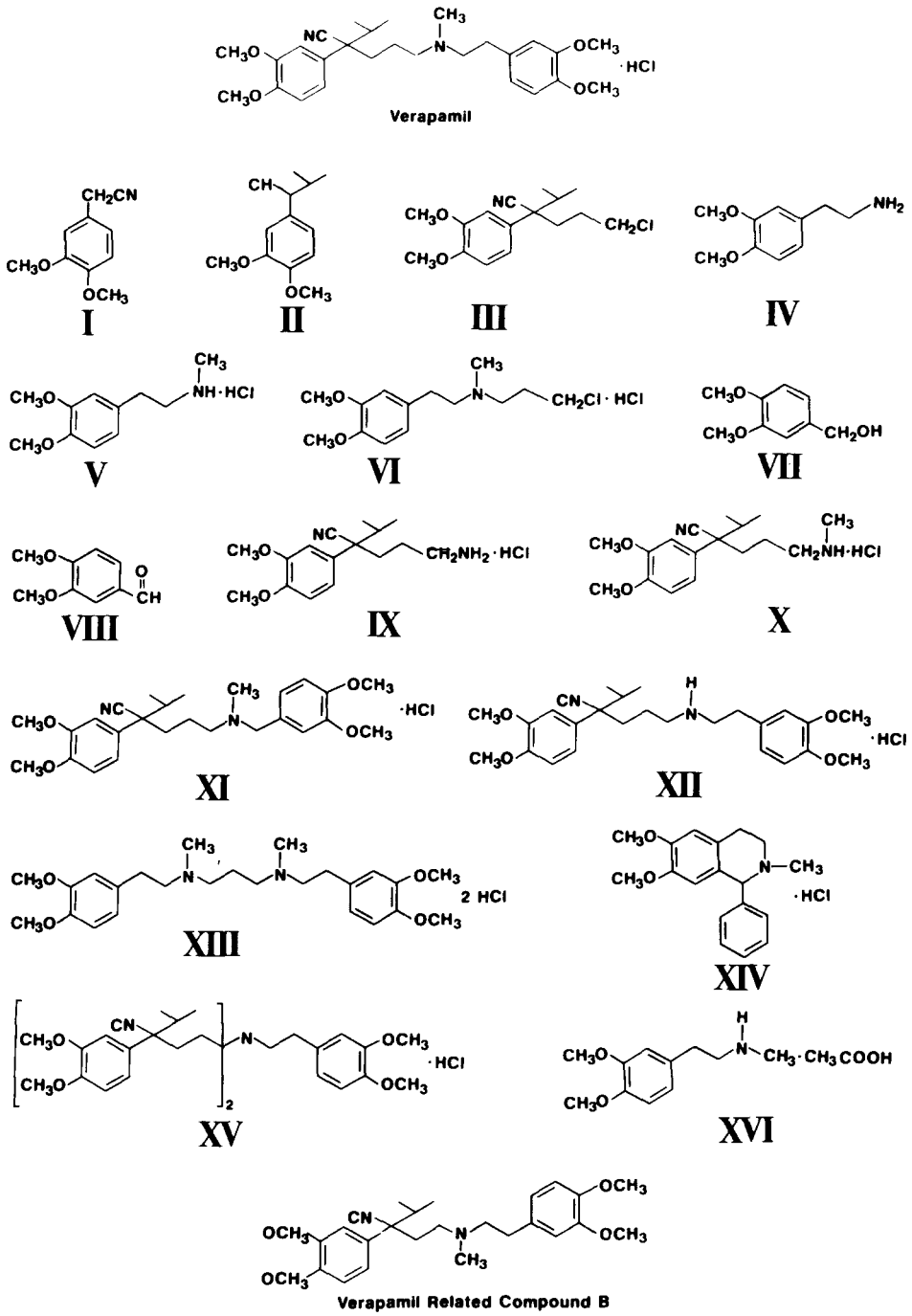


**Figure 1**
Chemical structures of verapamil and available related compounds.

*Apparatus*

The HPLC system (Model 2010, Varian) was fitted with a variable wavelength detector set at 278 nm (Varian Model 2050), an autosampler (WISP 710B, Waters, Milford, MA, USA) and an integrator (Perkin–Elmer LCI-100). The system was operated at room temperature with a flow rate of 0.9 ml $min^{-1}$. For method development, a Spherisorb ODS-2, 3 μm, 150 × 4.6 mm column (No. 088913) from Chromatography Sciences Company was used. A second column (No. 078922) from the same

**Table 1**
Chemical names of verapamil related compounds

| | |
|---|---|
| Verapamil HCl | Benzeneacetonitrile, α[-3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl) monohydrochloride |
| I | 3,4-Dimethoxyphenyl acetonitrile |
| II | 2-(3,4-Dimethoxyphenyl)-3-methyl butyronitrile |
| III | Benzeneacetonitrile-α-(3-chloropropyl)-3,4-dimethoxy-α-(1-methylethyl) |
| IV | 2-(3,4-Dimethoxyphenyl)ethylamine |
| V | *N*-methyl-2-(3,4-dimethoxyphenyl)ethylamine, hydrochloride |
| VI | *N*-methyl-*N*-(3-chloropropyl)-2-(3,4-dimethoxyphenyl)ethylamine, hydrochloride |
| VII | 3,4-Dimethoxybenzyl alcohol |
| VIII | 3,4-Dimethoxybenzaldehyde |
| IX | 2-Methyl-3-cyano-3-(3,4-dimethoxyphenyl)-6-aminohexane, hydrochloride |
| X (USP RS A) | 3,4-Dimethoxy-α-[(3-methylamino)-propyl]-α-(1-methylethyl) benzeneacetonitrile, monohydrochloride |
| XI | 1,6-Bis(3,4-dimethoxyphenyl)-methylaza-6-cyano-7-methyl-octane, hydrochloride |
| XII | 1,7-Bis(3,4-dimethoxyphenyl)-3-aza-7-cyano-8-methyl-nonane, hydrochloride |
| XIII | 1,3-Bis-[*N*-methyl-2-(3,4-dimethoxyphenyl) ethylamine]-propane, dihydrochloride |
| XIV | 1-Phenyl-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, hydrochloride |
| XV | *N*,*N*-di-*n*-propyl(α-isopropyl-3,4-dimethoxy-phenyl-acetonitrile)-3,4-dimethoxy-phenethyl-amine, hydrochloride |
| XVI | *N*-2-(3,4-dimethoxyphenyl)ethyl-*N*-methylamine acetate |
| USP RS B | Benzeneacetonitrile, α-[2-[[2-(3,4-dimethoxyphenyl) ethyl]-methylamino]ethyl]-3,4-dimethoxy-α-(1-methylethyl) monohydrochloride |

manufacturer and containing the same brand of packing material was used to evaluate column-to-column reproducibility.

*Mobile phase*

The eluent consisted of buffer–acetonitrile–2-aminoheptane (55:45:0.5, v/v/v) filtered through a 0.45 μm Nylon 66 filter. The buffer consisted of 0.01 M sodium acetate solution containing 33 ml $l^{-1}$ of glacial acetic acid.

*Solutions*

Verapamil hydrochloride reference standard and raw materials were dried at 105°C for 2 h prior to use. USP verapamil related compounds A and B were not dried. The following solutions were made up using the mobile phase: (1) resolution solution (0.01 mg $ml^{-1}$ verapamil hydrochloride, 0.01 mg $ml^{-1}$ USP verapamil related compound A and 0.01 mg $ml^{-1}$ USP verapamil related compound B); (2) standard solution (2.5 mg $ml^{-1}$ verapamil hydrochloride standard) and (3) test solution (2.5 mg $ml^{-1}$ verapamil hydrochloride).

*System suitability*

Six 10-μl aliquots of the resolution solution were injected into the chromatograph. The system was deemed to be suitable for use if the relative standard deviation of the verapamil peak response was not more than 5%, the resolution between verapamil and related compound A was not less than 8, the resolution between verapamil and related compound B was not less than 1.2 and the efficiency of the column, calculated using the verapamil peak was not less than 17 500 plates $m^{-1}$. The tailing factor for the verapamil peak, calculated using the USP formula, was not greater than 1.2. For drug assay, the relative standard deviation of six 10-μl aliquots of the standard solution was required to be not more than 0.6%. The retention times of related compound A, related compound B and verapamil were typically 2.8, 5.4 and 6.0 min, respectively.

*Procedure*

Ten microlitre aliquots of the resolution solution, standard solution and test solution were injected separately into the chromatograph and run for 45 min. The amount of each impurity in the test solution as a percentage of the total amount of drug was calculated using the formula $100(A_i/A_r)(C_r/C_u)$, where $A_i$ is the peak area due to the individual impurity, $A_r$ is the area of the verapamil peak in the resolution solution and $C_r$ and $C_u$ are the concentrations of verapamil hydrochloride in the resolution and the test solutions, respectively. The percentage of verapamil hydrochloride was calculated using the formula $100(A_u/A_s)(C_s/C_u)$, where $A_u$ and $A_s$ are the areas of the verapamil peak in the test and standard solutions, respectively, and $C_s$ and $C_u$ are the concentrations of verapamil hydrochloride in the standard and test solutions, respectively.

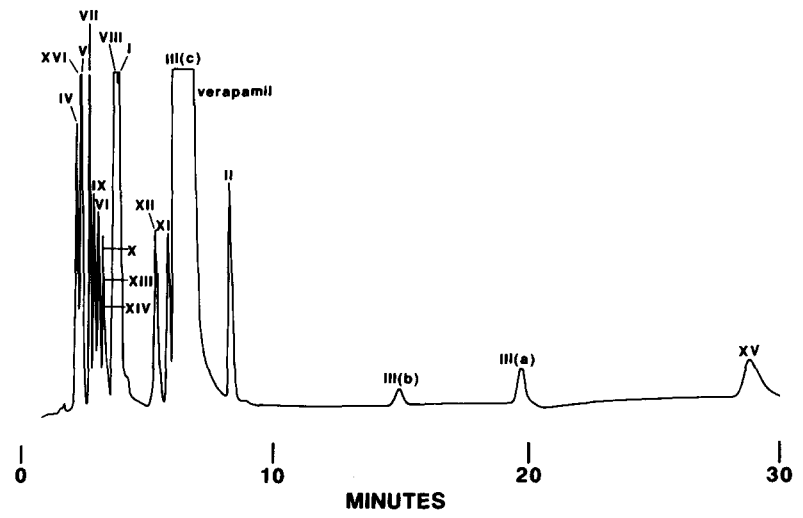


**Figure 2**
Chromatogram showing verapamil at a concentration of 2.5 mg $ml^{-1}$ and 16 related compounds, each at a concentration of about 0.01 mg $ml^{-1}$. Related compound **III** chromatographed as three peaks, labelled III(a), III(b) and III(c). Column, 150 × 4.6 mm i.d., 3 μm Spherisorb ODS-2 mobile phase, acetonitrile–0.01 M sodium acetate containing 33 ml $l^{-1}$ glacial acetic acid–2-aminoheptane (45:55:0.5, v/v/v).

## Results

Figure 2 shows the resolution of 16 related compounds from verapamil. All available related compounds were resolved from the drug, however, some compounds remained unresolved from each other. Impurity **V** and its acetic acid salt, **XVI**, eluted together. **X** and **XIII** were not resolved and **XIV** eluted on the tail of the composite peak of **X** and **XIII**, **XV** eluted after 30 min. The USP method also resolved all available related compounds from the drug, but compounds **IV**, **V**, **XIII**, **X** and **XVI** and the pair **VII** and **IX** were not resolved from each other.

*Linearity, sensitivity and precision*

The response of the HPLC system to verapamil hydrochloride and 16 related compounds was determined for concentrations ranging from the limit of determination to about 0.8% of the drug loading called for by the method. The limit of detection and limit of determination of each compound are presented in Table 2, along with results of the tests for linearity. The response of the HPLC system to verapamil at concentrations ranging from 50 to 150% of the assay range also was linear, $R^2$ = 0.994. Six replicate injections of the assay standard solution (verapamil hydrochloride, 2.5 mg $ml^{-1}$) were made on four different days over a period of 2 weeks. The relative standard deviation of the peak responses ranged between 0.11 and 0.43%. Debesis [10] proposes a maximum allowable RSD of 0.58% for an assay method requiring duplicate injections and limits of 98.5–101.5%.

*Stability of solutions*

Solutions of verapamil and the available related compounds were allowed to stand at room temperature for a period of 16 h. There were no visible signs of degradation. The apearance of additional peaks in the chromatograms was not observed and the HPLC area response of the original peaks remained constant for all the samples.

*Ruggedness*

Changing the buffer–acetonitrile ratio in the mobile phase from 55:45 to 60:40 (v/v) resulted in longer retention times; **XV** did not elute until 56.5 min. Changing the ratio to 50:50 (v/v) caused the retention times to decrease; **XI** was no longer resolved from verapamil and **II** eluted on the tail of the verapamil peak. Throughout method development, retention times on column 088913 remained constant. Another similarly packed column, No. 078922, was used to test column-to-column reproducibility. Although this column resolved **VIII** and **I** much better than the original column, resolution was lost between **XI** and **XII** and between **VI** and **IX**. The order of elution of **VI**, **VII** and **XIII** was different on these two columns. However, this did not interfere with drug assay or quantitation of the related compounds.

**Table 2**
Linearity and response data for verapamil and related compounds

| Compound | RRT* | L. Detection (%)† | L. Determination (%)‡ | Intercept (ct)§ | Slope × $10^{-6}$ ct/μg | RSQ‖ | Rel.¶ resp. |
|---|---|---|---|---|---|---|---|
| Verapamil | 1.00 | 0.01 | 0.02 | −1900 | 2.69 | 0.996 | 1.00 |
| **I** | 0.59 | 0.004 | 0.008 | 200 | 3.34 | 0.998 | 1.24 |
| **II** | 1.35 | 0.002 | 0.008 | −4300 | 2.87 | 0.995 | 1.07 |
| **III**** | 3.41 | 0.04 | 0.08 | −4800 | 1.53 | 0.986 | 0.57 |
| **IV** | 0.29 | 0.008 | 0.02 | 4400 | 3.25 | 1.000 | 1.21 |
| **V** | 0.31 | 0.008 | 0.02 | 5600 | 2.72 | 0.996 | 1.01 |
| **VI** | 0.44 | 0.008 | 0.02 | 3800 | 1.98 | 0.999 | 0.74 |
| **VII** | 0.38 | 0.008 | 0.02 | −16600 | 4.24 | 0.973 | 1.58 |
| **VIII** | 0.56 | 0.004 | 0.008 | 56100 | 14.5 | 0.981 | 5.39 |
| **IX** | 0.41 | 0.008 | 0.02 | 6400 | 2.16 | 0.997 | 0.80 |
| **X** | 0.47 | 0.004 | 0.008 | 2000 | 2.03 | 1.000 | 0.75 |
| **XI** | 0.91 | 0.008 | 0.02 | 2500 | 2.96 | 0.999 | 1.10 |
| **XII** | 0.83 | 0.008 | 0.02 | 2500 | 2.80 | 1.000 | 1.04 |
| **XIII** | 0.51 | 0.008 | 0.02 | 7400 | 2.17 | 0.994 | 0.81 |
| **XIV** | 0.53 | 0.008 | 0.02 | 1400 | 2.08 | 0.999 | 0.78 |
| **XV** | 5.39 | 0.02 | 0.04 | −14300 | 1.64 | 0.787 | 0.61 |
| **XVI** | 0.31 | 0.008 | 0.02 | 9000 | 2.48 | 0.999 | 0.92 |
| B | 0.91 | 0.008 | 0.02 | −7300 | 2.78 | 0.990 | 1.03 |

*Retention times relative to verapamil hydrochloride at about 5.78 min.
†Limit of detection is 2× baseline noise, on the basis of a 25 μg injection of verapamil hydrochloride.
‡Limit of determination is 4× baseline noise, on the basis of a 25 μg injection of varapamil hydrochloride.
§Area counts.
‖The square of the correlation coefficient.
¶Response relative to verapamil hydrochloride.
**Results are given for the component which produced the greatest response at 278 nm; this sample contained two other components eluting at RRT 1.12 and 2.56.

*Analysis of available products*

Impurity levels in six samples of verapamil hydrochloride raw material were determined. In all cases, total impurities were 0.3% or less and no single impurity exceeded 0.07%. The drug raw materials were assayed in triplicate. The means were between 99.7 and 100.5%, with relative standard deviations between 0.09 and 0.48%. The mean of six assays of the secondary standard used for the above analyses was 99.1% with a relative standard deviation of 1.4%, as determined against the USP reference standard.

## Discussion

All available related compounds eluted in about 30 min and compound **XV**, a potential by-product of the synthesis, could be determined down to levels of 0.04%. This compound had a retention time of about 200 min using the USP method which made it difficult to detect. Impurity levels were calculated by comparison to a verapamil standard and assuming the same sensitivity for the impurities as for the drug. This could lead to a slight overestimation of compounds **I**, **II**, **IV**, **V**, **VII**, **XI**, **XII** and compound B, and a slight underestimation of **III**, **VI**, **IX**, **X** and **XIII** to **XIV**. **VIII** could be over-estimated by over 400%. All of these compounds are considered ordinary impurities as defined by the USP, and as such do not require a more specific or accurate method of quantitation.

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