Impurity analysis of gabapentin by HPLC-UV-CAD

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Application benefits

- Simultaneous and sensitive detection of chromophore-deficient and volatile impurities with one single HPLC method instead of two separate methods, due to hyphenated detection techniques
- Shortened overall time of analysis and reduced consumption of consumables compared to two separate compendial methods
- Improved limits of quantitation (LOQs) for the chromophore-deficient impurities in accordance with the compendial requirements

Goal

This application aims to replace the two separate compendial methods for the impurity analysis of gabapentin employed in the European Pharmacopoeia (Ph. Eur.) with one single HPLC method with hyphenated ultraviolet and charged aerosol detectors (UV-CAD). The complementary hyphenated detection techniques will enable the simultaneous detection of analytes with divergent physico-chemical properties. The UV-CAD method meets the required LOQs for a compendial application.

Introduction

Gabapentin is commonly used for the treatment of focal seizures and, more recently, neuropathic pain. As its dosage may exceed an average daily intake of 2 g, regulatory authorities, such as the International Council on Harmonisation (ICH), impose strict requirements on the quality control of the drug by demanding a reporting threshold of 0.03% with respect to the concentration of main substance for possible impurities.[1] The requested

limits of quantitation (LOQs) cannot be accomplished when applying the routinely used HPLC-UV procedure, as gabapentin and some of its impurities are chromophore-deficient substances (Figure 1). Chromophore-deficient substances lack UV-absorbing structural elements, such as an aromatic ring or conjugated double bonds. The impurity profile of gabapentin entirely consists of compounds with saturated ring systems. Thus, the UV absorbance of the impurities, except for impurities A and D, is negligible. Quantitation of the impurities in the gabapentin monograph of the Ph. Eur. is accomplished by two separate HPLC-UV methods.[2] The compendial reporting threshold for possible impurities is therefore restricted to 0.05%, which contradicts the ICH directive.

The hyphenation of ultraviolet and charged aerosol detectors offers a solution to the challenging impurity profiling of gabapentin, since the complementary detection techniques enable the quantitation of all putative impurities with one single method at the required LOQs. In this application, we report a HPLC-UV-CAD method for the impurity analysis of gabapentin that allows simultaneous determination of the volatile impurity A and the chromophore-deficient impurities B, D, E, and G in one single chromatographic run at a quantitation limit of at least 0.03%.[3] Consequently, overall analysis time and costs are effectively reduced compared to the two separate compendial methods.

Experimental details

Chemicals

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (ACN) (P/N A955-212)
- Fisher Scientific™ Ammonium formate, Optima™ LC/MS grade (P/N A11550)
- Fisher Scientific™ Formic acid, Optima™ LC/MS grade (P/N A117-50)
- Gabapentin and impurities A, B, D, E, G were purchased from a reputable vendor

Sample handling

- Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer (P/N 14-955-152)
- Vials (amber, 2 mL), Fisher Scientific (P/N 03-391-6)
- Cap with Septum (Silicone/PTFE), Fisher Scientific (P/N 13-622-292)

Sample preparation

Stock solutions of the impurities A, E, and G were prepared by accurately weighing 10 mg of the respective impurity and dissolving it in 10.0 mL deionized water. Stock solutions of the impurities B and D were prepared accordingly, except for dissolution in methanol. The stock solutions were used for preparing the calibration standards and for spiking of the sample solutions by appropriate dilution with mobile phase A. The stock solutions were stored at 8 °C (-20 °C for impurity B) and were stable for at least one week.

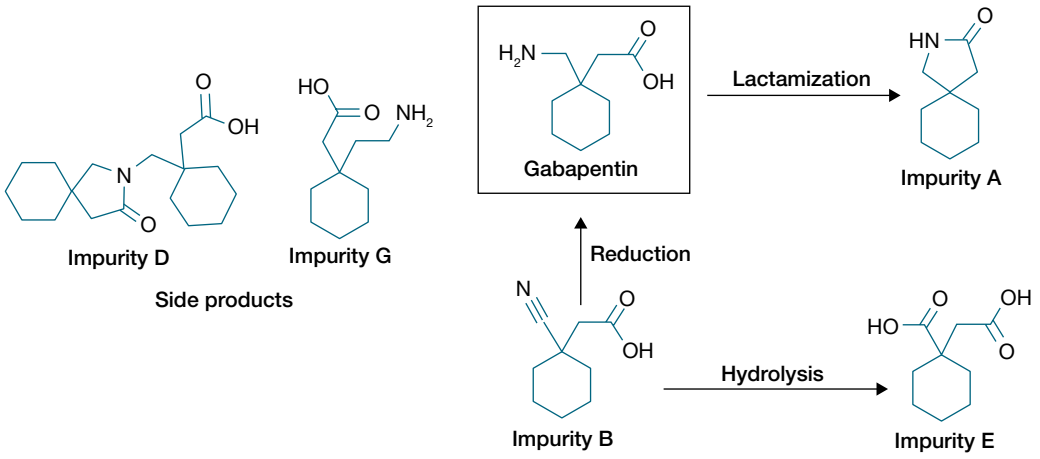

Figure 1. Impurity profile of gabapentin with respect to the Ph. Eur. 10.3[2]

Sample solutions were freshly prepared daily by weighing 80 mg of gabapentin and dissolving in 10.0 mL mobile phase A. The sample solutions were stable for at least one day at room temperature.

Instrumentation

- Thermo Scientific™ Vanquish™ Flex Binary UHPLC system consisting of:
  - Vanquish System Base (P/N VF-S01-A-02)
  - Vanquish Binary Pump F (P/N VF-P10-A)
  - Vanquish Split Sampler HT (P/N VH-A10-A)
  - Vanquish Column Compartment H (P/N VH-C10-A-02)
  - Vanquish Variable Wavelength Detector F (P/N VF-D40-A)
  - Standard flow cell, biocompatible, 11 µL (P/N 6077.0200)
  - Vanquish Charged Aerosol Detector H (P/N VH-D20-A)

Gabapentin method

Table 1. Chromatographic conditions

Parameter	Value
Column	Agilent™ Zorbax™-SB C8 (250 × 4.6 mm, 5 µm)
Mobile phase	A: 20 mM Ammonium formate pH 2.8 in water B: 20 mM Ammonium formate pH 2.8 in water/ACN 10/90 (v/v)
Gradient	*Time (min)* *%B* 0–1 25 1–5 25–60 5–11 60 11–12 60–25 12–15 25
Run time	15 min
Flow rate	1.2 mL/min
Column temperature	25 °C (still air mode)
Autosampler temperature	8 °C
Autosampler wash solvent	Methanol
Injection volume	20 µL
Detector settings (CAD)	Evaporation temperature: 30 °C Power function value: 1.3 Filter constant: 5 s Data collection rate: 10 Hz
Detector settings (UV)	Detection wavelength: 210 nm Data collection rate: 20 Hz Response time: 0.20 s

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data system (CDS), version 7.2.6 was used for data acquisition and analysis.

Results and discussion

Method development

The polarity of the zwitterionic γ-aminobutyric acid derivative gabapentin is higher compared to its increasingly alkyl-substituted impurities (Figure 1). Since all analytes share a cyclohexyl group as part of their molecular structure, they can be separated by reversed-phase (RP)-HPLC. Stationary phases of moderate hydrophobicity, such as C8, phenyl, or biphenyl, represent a good compromise for the separation of gabapentin and its medium hydrophobic impurities when applying reversed-phase RP-HPLC. Various C8 and phenyl columns were tested for their separation efficacy toward the analytes. A C8 column was selected for further method development that provided the best resolution for the critical peak pair of impurities B and A and, in addition, adequate peak shapes for all analytes. The pH of the ammonium formate buffer used in the method was adjusted with formic acid to 2.8 to suppress deprotonation of the acidic analytes, resulting in improved peak shapes and prolonged retention times of the latter. Using a 20 mM ammonium formate buffer pH 2.8 in a mixture of water/ACN resulted in sharp gaussian peak shapes for all analytes. To facilitate the elution of the most hydrophobic impurity D subsequent to the other analytes in one chromatographic run, a gradient program had to be applied. The flow rate of the pump was set at 1.2 mL/min resulting in a relatively short run time of 15 min including re-equilibration time. The instrumental settings of the CAD were optimized toward sensitive detection of semi-volatile impurity B. Adjustment of the CAD's evaporation temperature (30 °C) and filter constant (5 s) settings enabled the accurate quantitation of semi-volatile impurity B at the required reporting threshold (0.03%).

UV-CAD detection

As gabapentin itself and the impurities B, E, and G lack suitable chromophores in their structures, applying low wavelength UV detection <220 nm is not sufficient to compensate the structural deficits. A method solely based on UV detection suffers from poor sensitivity. This issue can be overcome by employing a CAD for the determination of the chromophore-deficient impurities B, E, and G. However, a sensitive quantitation of impurity A is not possible with CAD due to the compound's relatively high volatility.

Hyphenation of UV and CAD is beneficial in this case, since impurity A can be detected by UV with sufficient sensitivity, while the remaining chromophore-deficient impurities are subsequently covered by CAD. The in-line coupling of the complementary detectors thereby enabled simultaneous quantitation of all putative impurities at the ICH claimed reporting threshold of 0.03% being equivalent to 50 ng on column injected mass. Excellent LOQs (<10 ng injected mass) were obtained for the non-volatile impurities, while the method was sufficiently sensitive toward the UV detected impurity A (50 ng) and the semi-volatile impurity B (50 ng) (Table 2).

Method validation

The method was validated with respect to the requirements outlined in ICH guideline Q2(R1).[4] Specificity of the method was demonstrated by injection of a sample solution spiked with 0.1% of each impurity (Figure 2). The resolution was >1.5 for all peak pairs, proving adequate selectivity. Linearity was evaluated over a concentration range from 0.03 to 0.24%. Calibration curves for the impurities were established by linear regression of six equally distributed calibration levels. The coefficients of determination (R^2) were >0.999 (Table 2) and the residual plots showed evenly scattered calibration points indicating a good quality of fit. Accuracy and precision were both evaluated on spiked sample solutions. The % recovery rates obtained from calculation by linear regression and the %RSD for

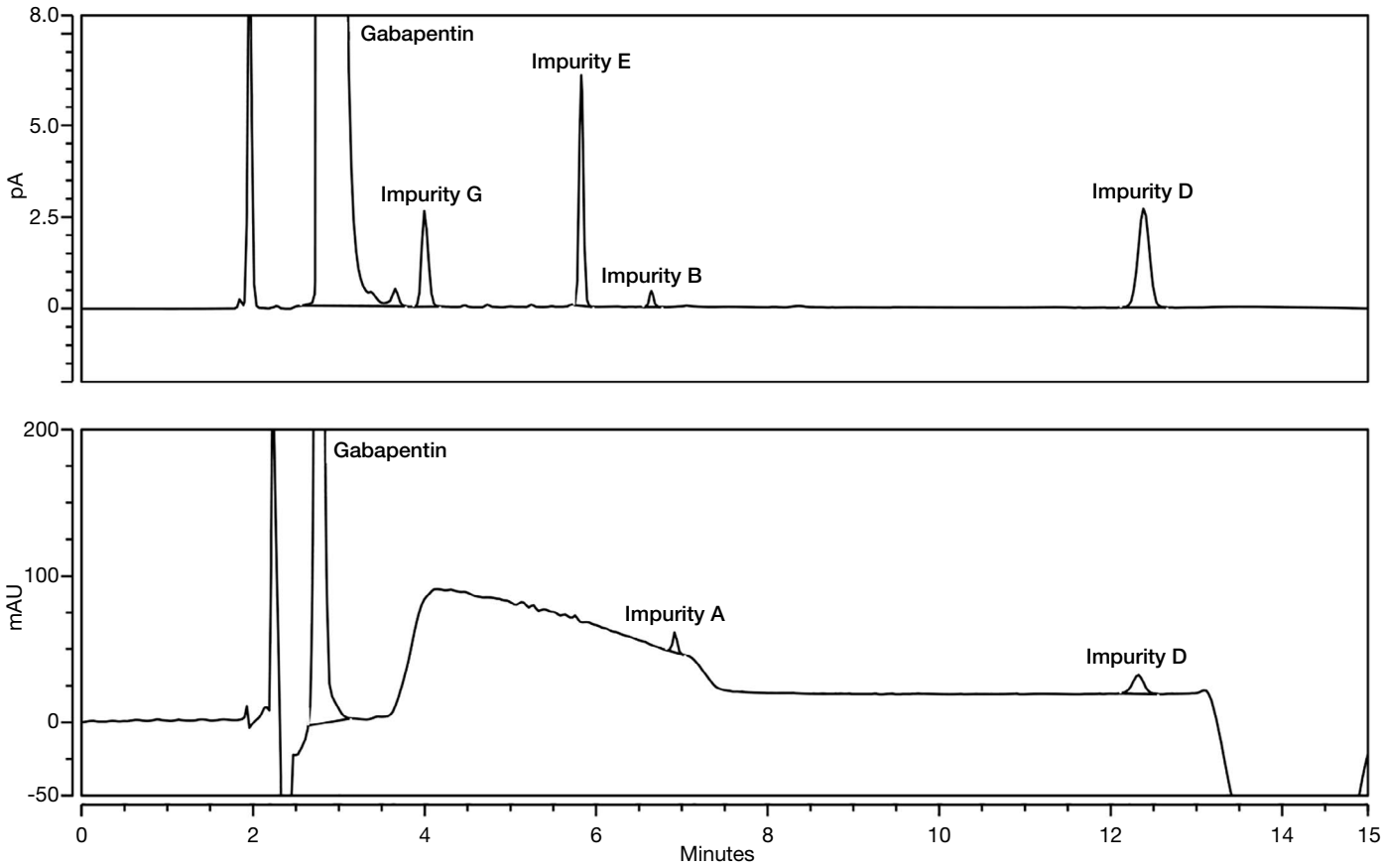

Figure 2. Chromatograms of the CAD (upper chromatogram) and UV signal for a gabapentin sample solution (8 mg/mL) spiked with 0.1% of each impurity

Table 2. Validation results of the gabapentin method

Parameter	Condition/ concentration	Gabapentin	Impurity A[a]	Impurity B	Impurity D	Impurity E	Impurity G
Linearity	Equation[b] y = mx + t	0.0017× - 0.0032	0.0064× - 0.0145	0.00017× - 0.0041	0.0024× - 0.0213	0.0021× - 0.0164	0.0013× - 0.0052
	R^2	0.9995	0.9996	0.9993	0.9999	0.9995	0.9996
	SD slope [pA/ng]	1.8×10^{-5}	6.5×10^{-5} [a]	2.3×10^{-6}	1.3×10^{-5}	2.4×10^{-5}	1.2×10^{-5}
	SD intercept [pA]	0.0048	0.018[a]	0.00057	0.0034	0.0065	0.0033
Accuracy (% recovery rate)	0.03%, n=3	n.d.[c]	99	98	102	105	98
	0.10%, n=3	n.d.	n.d.	93	102	103	97
	0.20%, n=3	n.d	n.d.	112	102	101	99
LOQ	Inject. mass (ng)	3	50	50	4	2	3
LOD	Inject. mass (ng)	1	n.d.	n.d.	2	1	1
Intraday precision	0.03%, n=6	n.d.	2.7[d]	8.4	2.1	3.1	2.2
	0.10%, n=6	n.d.	1.3	7.7	1.2	1.6	1.6
	0.20%, n=6	n.d.	0.8	5.4	1.0	1.4	1.1
Interday precision	0.03%, n=2	n.d.	3.8	8.2	1.9	2.8	4.7
	0.10%, n=2	n.d.	1.1	6.0	1.2	2.0	2.4
	0.20%, n=2	n.d.	0.9	4.6	1.1	1.5	1.6

[a] Detected by UV, SD slope [mAu/ng], SD intercept [mAU].
[b] Obtained from linear regression of the 6 calibration points.
[c] Not determined.
[d] %RSD

intra- and inter-day repeatability are illustrated in Table 2. With recovery rates ranging from 97 to 105% and repeatability %RSD values ranging from 0.8 to 4.7% for the non-volatile impurities, the method can be considered as sufficiently accurate and precise for the intended purpose. The recovery rates (93–112%) as well as the repeatability %RSD (4.6–8.4%) were slightly increased for semi-volatile impurity B compared to the non-volatile analytes. However, the results were still within an acceptable range. The limits of detection (LOQs) of the method were calculated using the S/N approach of ICH guideline Q2(R1)[4] (Table 2). LOQs of ≤50 ng injected mass were achieved for the impurities, which enabled accurate quantitation at the required reporting threshold of 0.03%. For evaluation of the method's robustness, the resolution for each peak pair was monitored against small variations of flow rate (1.1–1.3 mL/min), temperature of the column chamber (20–30 °C), initial % of mobile phase B (23–27%), and final % of mobile phase B (60–62%). The method can be regarded as robust against these small changes.

UV-CAD method vs. compendial procedures

The most evident benefit of the UV-CAD method over the two separate compendial methods employed in the Ph. Eur.[2] is the improved sensitivity for the chromophore-deficient analytes. Sample concentration was reduced from 14 mg/mL in the compendial method to 8 mg/mL in the UV-CAD methods, accompanied with a lowered reporting threshold. Consequently, the general reporting threshold for the impurities can be reduced from 0.05 to 0.03% to comply with the requirements of ICH guideline Q3A (R2)[1] for drugs with an average daily intake >2 g. Moreover, combining two separate methods with a respective run time of 15 min to one single method with just 15 min run time substantially shortens the overall analysis time and reduces the number of required consumables and instrumentation.

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Conclusion

- A simple, rapid, and selective HPLC-UV-CAD method for the impurity profiling of gabapentin was developed.
- Hyphenation of UV and CAD enabled the simultaneous detection of volatile impurity A and the chromophore-deficient impurities B, D, E, and G in one chromatographic run.
- Compared to the two separate compendial methods employed in the Ph. Eur., the reporting threshold was improved, besides time and costs savings by reducing the mobile phase and column consumption.

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