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Journal of Pharmaceutical and Biomedical Analysis 37 (2005) 529–534
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Determination of topiramate and its degradation product in liquid oral solutions by high performance liquid chromatography with a chemiluminescent nitrogen detector

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Received 20 January 2004; received in revised form 5 November 2004; accepted 6 November 2004
Available online 19 December 2004

Abstract

Topiramate is a sulfamate-substituted monosaccharide that is prescribed for the treatment of epilepsy. It has been a challenge to develop analytical methods for topiramate formulations because the compounds of interest do not have chromophores that are active above 190 nm and because of interference from excipients. This paper describes a simple, specific, precise, accurate, and sensitive method using a chemiluminescent nitrogen detector. The method has a validated linearity range of 32–4800 ng of topiramate and excellent precision (system repeatability). The limit of quantitation was determined to be 0.1% for the degradation product w/w versus topiramate. The method has been successfully used for probe stability studies in support of early phase formulation development.
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Keywords: Chemiluminescent nitrogen detector; High-performance liquid chromatography; Topiramate; Method development and validation

1. Introduction

Topiramate (TOPAMAX®) is chemically 2,3:4,5-bis-*O*-(1-methylethylidene)-β-D-fructopyranose sulfamate, a sugar derivative intended for use as an antiepileptic drug. It is available as tablets for oral administration and sprinkle capsules that can be opened and sprinkled onto soft foods. Like most other carbohydrates and their derivatives, topiramate and its degradation product do not have chromophores that are active above 190 nm (Fig. 1). As a result, the most widely used UV–vis detectors cannot be used for quantitative analysis. Instead a refractive index (RI) detector has to be used. Topiramate solid dosage forms also contain inactive ingredients such as lactose monohydrate, pregelatinized starch, microcrystalline cellulose, sodium starch glycolate, hydroxypropyl methylcellulose, and polyethylene glycol. These materials are either insoluble in aqueous solutions or not retained on regular reversed-phase HPLC columns. This allows the quantitation of topiramate and degradation product with no significant interference from the excipients even though a universal RI detector is used.

However, the RI detector was not applicable for analysis of topiramate oral liquid formulations, which have been under development by Johnson & Johnson Pharmaceutical Research & Development (J&JPRD), because they contain large quantities of water-soluble excipients that were added to enhance solubility of the active pharmaceutical ingredient (API). A chromatogram of such a sample obtained on an RI detector is shown in Fig. 2. In support of the formulation development, the method development chemists were challenged to identify a suitable method for the quantitative analysis of topiramate and degradation product in the liquid formulations.

Several technologies were considered for the purpose [3,4]. A capillary gas chromatography method with flame

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doi:10.1016/j.jpba.2004.11.010

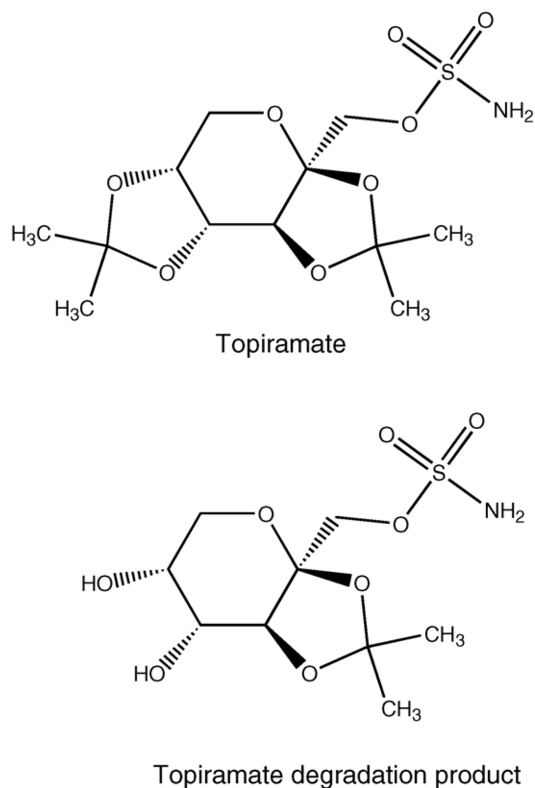
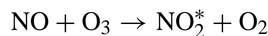


Fig. 1. Structures of topiramate and its degradation product.

ionization detection was evaluated. It was found that the topiramate degradation product (Fig. 1) was not thermally stable for GC analysis. A reversed-phase HPLC method coupled with an evaporative light scattering detector (ELSD) also was evaluated. The results demonstrated that the ELSD was suitable for quantitative analysis of the API but not for the low-level degradation product. We finally decided to explore the use of a chemiluminescent nitrogen detector (CLND) along with reversed-phase HPLC for method development.

The working principle of the CLND is based on the following reactions:



$$NO + O_3 \rightarrow NO_2^* + O_2$$

$$NO_2^* \rightarrow NO_2 + h\nu$$

where $h\nu$ is the electromagnetic radiation. In this reaction, nitric oxide (NO) reacts with ozone to form nitrogen dioxide (NO_2) in an excited state, which emits photons when returning to the ground state. This reaction was thoroughly studied in the 1960s [1,2].

In 1970, Fontijn et al. [5] first reported the use of this chemistry for quantitative analysis of air pollutants. In their research, the polluted air (containing NO) and ozone were diffused into a continuous flow reaction chamber that was kept at a vacuum of 1 Torr and ambient temperature. Ozone was introduced to the chamber at large excess. The emitted light intensity was measured by a photomultiplier tube. A linearity range of 4 ppb to 100 ppm for NO was reported. The first CLND was developed as a detector for GC in the early 1990s [6]. To couple the detector with HPLC, two major problems had to be solved. The first was the stoichiometrical conversion of the covalently bonded nitrogen in the analytes into NO. The second was the elimination of water from the reaction gas to prevent reaction with ozone. The first problem was solved by using a nebulizer and a high-temperature pyrolysis furnace. It was found that

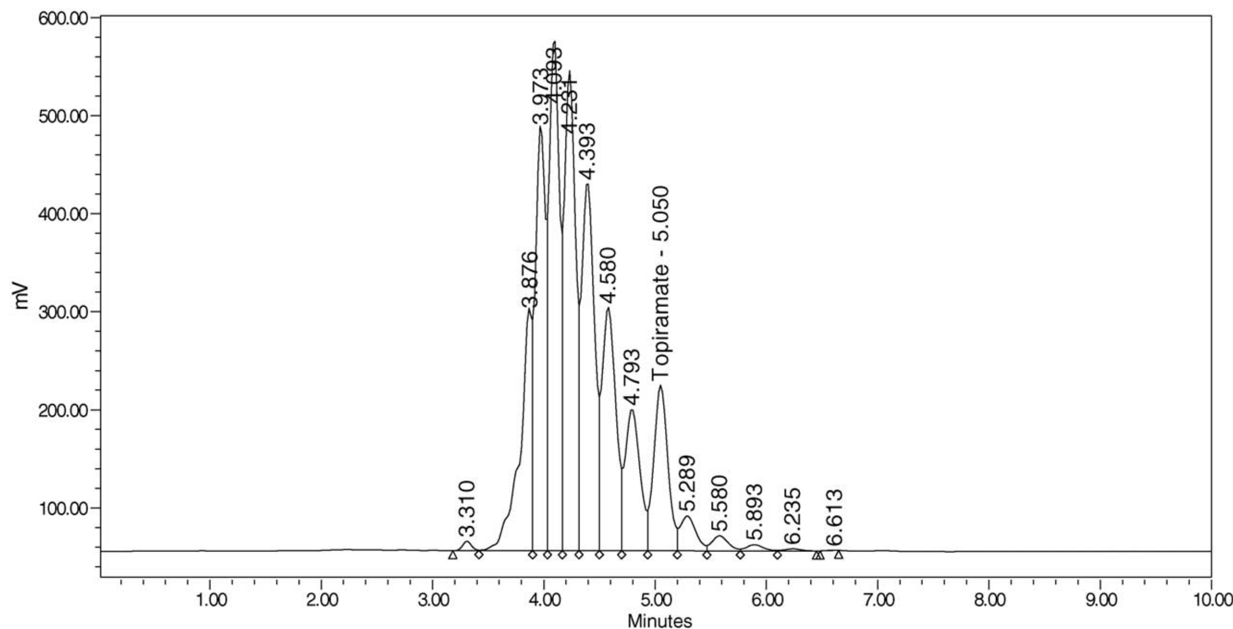


Fig. 2. Chromatogram of a topiramate oral liquid sample using an RI detector.

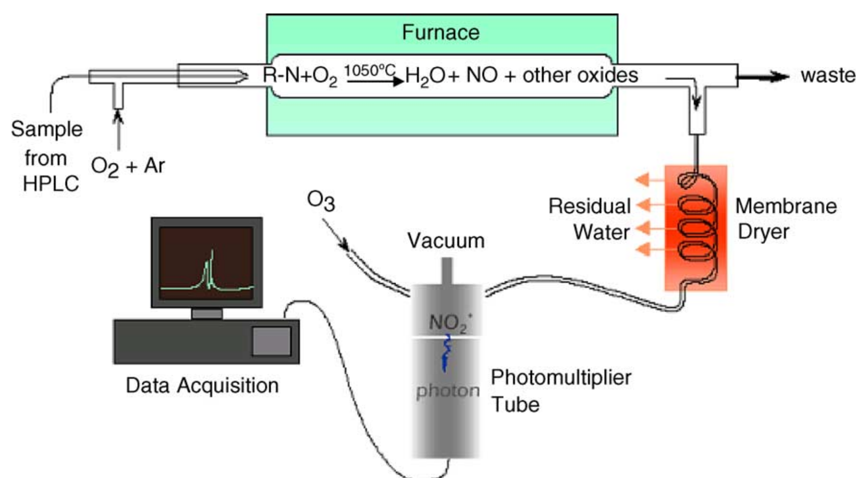


Fig. 3. Schematic diagram of the CLND detector.

keeping the pyrolysis furnace temperature above 1000 °C quantitatively converts the nitrogen into NO. The second problem was solved by the introduction of a membrane dryer [7]. A schematic diagram of the CLND is shown in Fig. 3.

The CLND is a highly sensitive and selective detector. It has also been reported to have equimolar responses regardless of the chemical state of nitrogen [8]. This combination of characteristics can be very useful in pharmaceutical analysis, particularly in early phase impurity profiling and degradation studies. In early phases of pharmaceutical development, process impurities and degradation products are not well defined and characterized. Their detection and quantitation usually are based on area percent estimation using a UV detector. This may introduce a large error in quantitation of these impurities because of a large variation in molar extinction coefficients. There may be impurities that are not detected due to the lack of active chromophores. Use of the CLND as a complimentary method along with a UV detector will make detection and quantitation of impurities more accurate and complete [17].

The CLND has been interfaced with HPLC, GC, CE and SFC [9–12]. It has been used in applications in combinatory chemistry to characterize combinatorial libraries as a complementary method [13]. It has also been used in analysis of amino acids, and peptides [14], as well as nucleotides and nucleosides in the food industry [15]. However, we have seen few, if any, applications using CLND as the primary method for pharmaceutical analysis. In this paper we describe a simple, accurate, precise, and sensitive HPLC method interfaced with the CLND for quantitative analysis of topiramate and its degradation product. Some aspects of method development and validation will be discussed. Data will be presented to demonstrate the successful use of the method for supporting probe stability studies in formulation development.

2. Materials and methods

2.1. Chemicals and reagents

HPLC grade methanol was purchased from EM Science (An affiliate of Merck KGaA, Darmstadt, Germany). HPLC grade equivalent water was obtained from an in-house Millipore Milli-Q-Gradient ultrapure water system (Millipore, USA). The argon that was used for the nebulizer and oxygen that was used for the nebulizer, pyrolysis furnace, and ozone generator were purchased from Airgas (Radnor, PA). This study also involves proprietary topiramate oral liquid formulations under development by J&JPRD, which have strengths in the range of 10–30 mg/mL.

2.2. Chromatographic conditions

A Model 8060 CLND from Antek Instruments (Houston, TX) and an Agilent 1100 series HPLC system (Wilmington, Delaware) was interfaced through an Accurate ICP-04-20 flow splitter with a split ratio of 1:4 from LC Packings A Dionex (The Netherlands). The CLND has been equipped with the new ceramic pyrolysis furnace. To avoid noisy baselines, a new in-line degasser module was purchased from Agilent to replace the old one that had been in contact with acetonitrile.

The chromatographic separation of topiramate from the degradation product was achieved using a 250 mm × 4.6 mm Keystone Phenyl/B, 5 μm particle size column from Thermo Electron Corporation (Bellefonte, PA). The mobile phase was 65% methanol in water (v/v) with a flow rate of 0.8 mL/min. By using the flow splitter, the actual mobile phase entering the pyrolysis furnace was at 0.20 mL/min. The injection volume was 5 μL for assay of topiramate and 50 μL for assay of degradation product.

The CLND parameters were set as follows: pyrolysis furnace and membrane dryer temperature at 1050 and 85 °C,

respectively; oxygen, argon, make up, and ozone flow rates at 250, 150, 50 and 25 cm^3/min, respectively.

2.3. Preparation of solutions

The mobile phase was used as a sample solvent. The standard solutions were prepared to contain about 1.6 mg/mL of topiramate reference standard. The sample solutions were prepared by transferring a suitable amount of the oral liquid into a suitable size volumetric flask and dissolving in sample solvent to obtain the same concentration of topiramate as the standard solutions.

3. Results and discussion

3.1. Method development

In this study, the CLND was chosen as the method for supporting early phase formulation development of topiramate. The HPLC part of the method consists of a reversed-phase phenyl column and a methanol/water mobile phase. Based on the previous degradation studies, only one degradation product was to be monitored. The separation of topiramate from the degradation product was easily achieved. Adoption of the CLND eliminated all interferences from the placebos of the liquid formulations because they do not contain nitrogen. The method development work focused on optimizing operating conditions for the detector. It should be pointed out that topiramate may have different degradation pathways in solution formulations compared with solid dosage forms. To ensure the detection of unknown degradation products, an orthogonal method has been implemented to look for different degradation products.

Based on the instrument manufacturer's specifications, the CLND has a linear range between 0.3 and 3000 ng of nitrogen, corresponding to 7–70,000 ng of topiramate. By using the aforementioned HPLC conditions, a 10 μL injection of the 0.016 mg/mL topiramate solution gave a detectable signal ($S/N \geq 3$). The amount of topiramate introduced into the detector was equivalent to 32 ng of topiramate or 1% of the nominal concentration (1.6 mg/mL). To achieve a quantitation limit of 0.1% for the degradation product, a 5–10 times increase in injection amount of topiramate is needed. Theoretically, this may be achieved by increasing the sample concentration and/or the injection volume. But either way, it may cause column overloading. It may also cause problems with the detector, such as incomplete combustion that can lead to carbon deposits inside the pyrolysis furnace and membrane dryer. To avoid these problems, we took advantage of the built in flow switching capabilities of the Agilent 1100 HPLC system. To improve sensitivity for the degradation product, the injection volume was increased to50 μL. In the time window when the AIP was eluted, the eluent was switched to waste automatically to avoid overloading the detector. Typical chromatograms of a sample are presented in Fig. 4. This approach has worked effectively for assay of topiramate and degradation product in the oral liquids.

3.2. Method validation

The method validation work was conducted according to the pre-established company guidelines, which are based on the regulatory requirements [18–21]. The development phase of the project also dictated the selection of validation parameters, which included specificity, accuracy, precision (system repeatability), linearity, sensitivity (limit of quantitation), and solution stability. Specificity of the method was demonstrated by showing no interference from a blank or placebo. Separation of topiramate and its degradation product in standard and sample solutions was also demonstrated. The following sections discuss the majority of the validation results.

3.2.1. Linearity

The linearity of the method was evaluated for topiramate by preparing a series of standard solutions in the range of 0.016–2.36 mg/mL, corresponding to 1–150% of the nominal concentration. These solutions were injected and peak areas were obtained and plotted. The linearity plot had a slope of 712,000, an intercept of 5200 and the square of the correlation coefficient was 0.9998, indicating excellent linearity.

3.2.2. Precision (system repeatability) and accuracy

Because this is an early phase analytical method, only the system repeatability was evaluated. Modern HPLC systems equipped with the widely used UV and RI detectors offer excellent system repeatability. The % R.S.D. of the responses (i.e. peak areas) from multiple injections of a solution of suitable concentration can be less than 0.5% [16]. The CLND is not as rugged as the typical UV and RI detectors. Inconsistent performance from any part of the detector, including the nebulizer, pyrolysis furnace, membrane dryer, and vacuum control, may result in poor system repeatability.

The precision study was conducted by injecting 10 times each topiramate standard solutions at 0.5, 0.9 and 2.0 mg/mL. The peak areas at each concentration from the 10 injections had % R.S.D.s of 0.7, 0.9 and 0.3%, respectively. The results indicate that the CLND will not cause significant deterioration in system repeatability when it is working properly. On the other hand, a large % R.S.D. is an indication of detector malfunctioning rather than injector or chromatography related problems most of the time.

The accuracy study was conducted by spiking the placebo with topiramate at three different levels. The details are presented in Table 1.

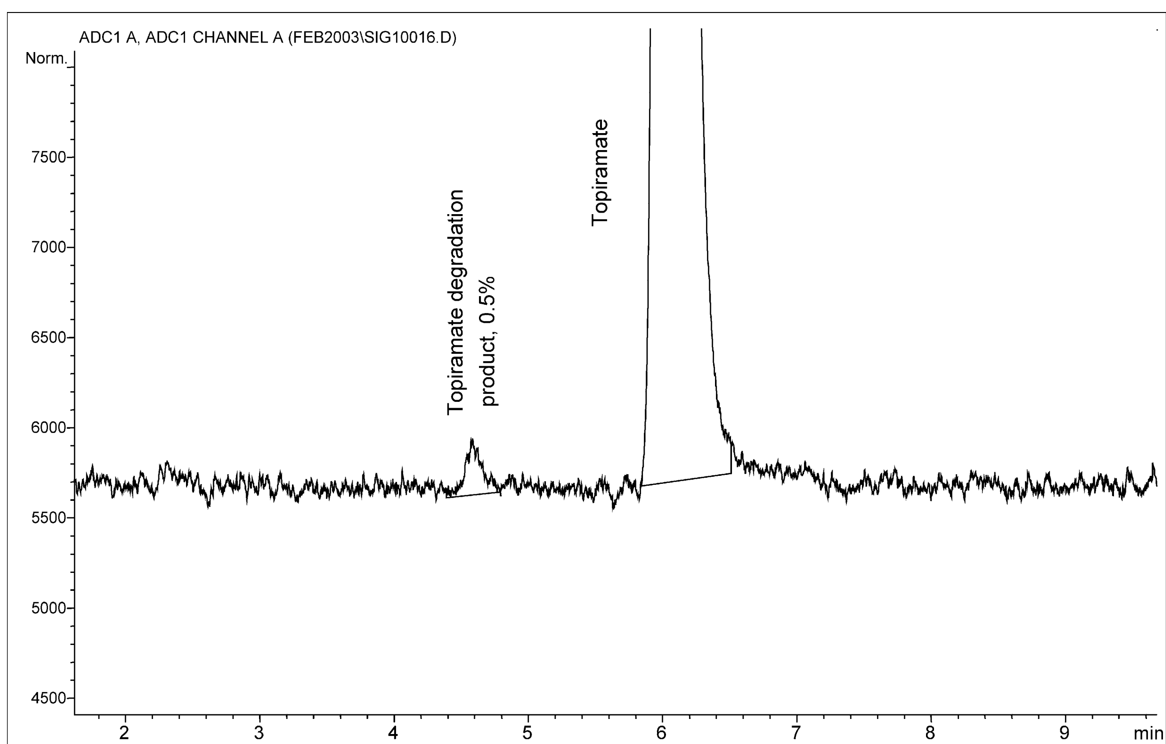
3.2.3. Limit of quantitation (LOQ)

The regulatory guidelines require the quantitation of unspecified impurities at the 0.1% level. The limit of detection

of this method for topiramate-related compounds was determined to be 0.05% a S/N ratio of 3. The LOQ of this method for topiramate and its degradation product was estimated using the signal-to-noise-ratio approach. It was estimated to be 0.1% at a S/N ratio of 10, which was appropriate for this early phase method.

3.2.4. System suitability

For this method, system suitability requirements are set to ensure system repeatability and sensitivity. In every sequence of sample analyses, five injections of standard are made first and the obtained peak areas are used for calibration. The % R.S.D. of the responses from the standard injections should



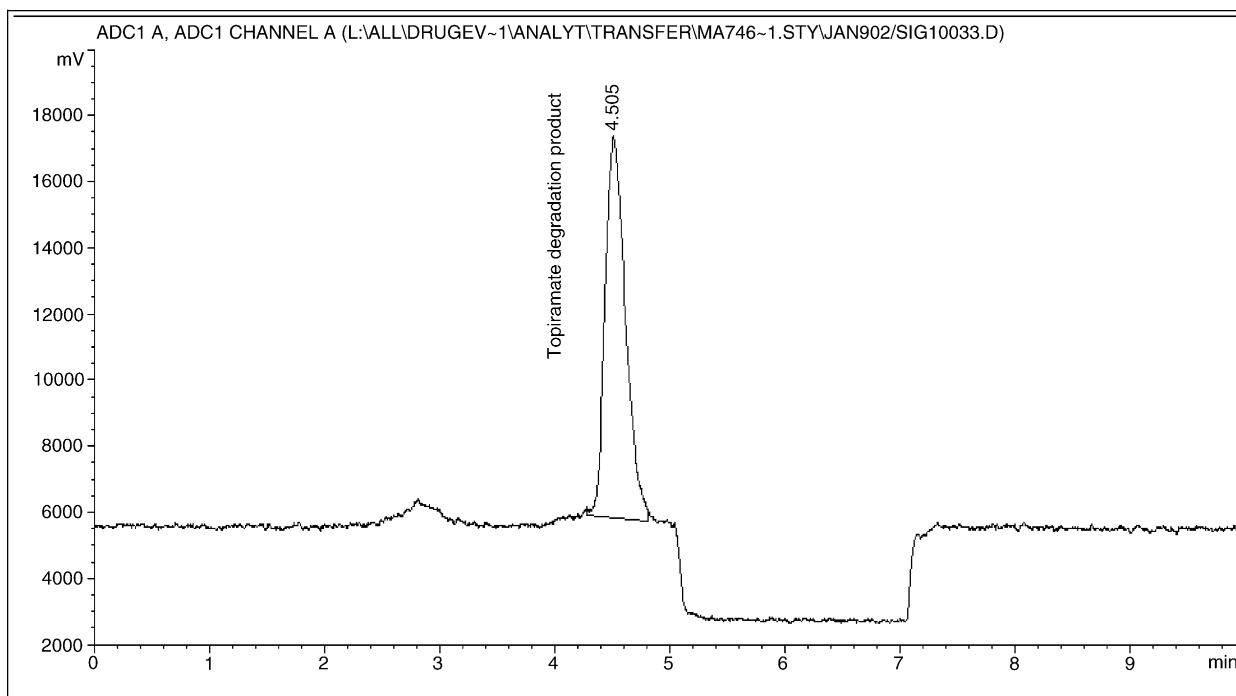


Fig. 4. Chromatograms of topiramate oral liquid sample using a CLND detector.

Table 1
Accuracy and analysis repeatability

Level (% w/w)	Amount DS added (mg)	Placebo added (mL)	DS conc. (mg/mL)	Recovery (%)
80	12.38	3.0	1.2368	99.6
100	16.23	3.0	1.6214	100.2
100	16.58	3.0	1.6563	100.6
100	16.03	3.0	1.6014	100.7
120	19.76	3.0	1.9740	101.8
Mean				100.6
% R.S.D.				0.8

be ≤ 2.0. Following each set of up to 10 samples, a check standard is injected and must assay at 98.0–102.0%. If one of the check standards fails the system suitability, results for the samples injected after the failed check standard are considered invalid. In every sequence, a sensitivity check standard at 0.1% of the nominal concentration is injected to ensure sensitivity. The signal-to-noise ratio must be greater than or equal to 10.

4. Conclusion

The results presented in this paper demonstrate that the CLND can be used to develop primary assay methods in support of early phase pharmaceutical development. The performance of the detector, demonstrated by accuracy, precision and sensitivity of the method, meets all requirements. In addition, its high selectivity for nitrogen-containing compounds is a valuable advantage, simplifying method development for complex formulations. However, it should be pointed out that we have experienced higher frequencies of hardware breakdowns compared with regular UV and RI detectors, including clogged nebulizer, broken pyrolysis furnace and dirty membrane dryer. The system suitability criteria (Section 3.2.4) have ensured the performance of this method. The availability of reliable service from the instrument vender also is important to ensure the routine use of this detector for stability and release testing of the experimental formulations of topiramate.

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