Development of a stability-indicating method for esomeprazole and related degradation products by automated method scouting and mass detection

Authors: Soo Hyun Park, Sylvia Grosse, Mauro De Pra, Michaela Scherz, Frank Steiner

Thermo Fisher Scientific, Germering, Germany

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Application benefits
- Thermo Scientific™ Vanquish™ Method Development UHPLC system in combination with ChromSword Chromeleon Connect, enables automated and unattended method development, reducing method development time significantly.

- ChromSword Chromeleon Connect accelerates the screening of column and mobile phases for the determination of esomeprazole and related degradation products.

- Coupling a Thermo Scientific™ ISQ™ EM single quadrupole mass detector to a Vanquish UHPLC Method Development system increases peak tracking capability in method development by the combined use of mass and UV spectra.

Goal
Demonstrate a streamlined method scouting workflow for the analysis of esomeprazole and related degradation products.

Introduction
The development of a stability-indicating HPLC method is an essential analytical task in the pre-clinical phases of drug development. Every drug candidate undergoes stability studies during its development and selection. The method must be capable of chromatographically resolving the expected impurities resulting from the degradation of the active pharmaceutical ingredient (API). Based on these requirements, the development of such methods can be a demanding and time-consuming task.

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A systematic approach in HPLC method development is essential to decrease development time and resulting costs. Method scouting is typically performed prior to optimization to select the most promising chromatographic conditions. The chromatographic parameters generally screened during method scouting are column type, mobile phase buffer compositions and pH, and different organic solvents. Screening several candidate methods is common practice in pharmaceutical labs where, for example, several different stationary phases are screened at different mobile-phase compositions and buffer pH with consideration of maximal practical peak capacity.[1] Automation in method scouting accelerates overall method development, requiring advanced hardware technology and intelligent software. For example, systems for automated method development include two column selection valves and a solvent selection valve, to enable automatic selection (and switching) of up to six columns and 13 different solvents. The Scout module of ChromSword Chromeleon Connect software runs scouting experiments using different combinations of columns and mobile phases in an unattended manner, based on a sequence created very quickly and intuitively.

Esomeprazole is the S-enantiomer of omeprazole and belongs to a class of drugs called proton pump inhibitors (PPI), which reduce stomach acid by the inhibition of the gastric H^+/K^+ APTase in parietal cells. Esomeprazole is widely used in treatments of gastroesophageal reflux disease including erosive esophagitis, duodenal ulcer, and other gastric acid related diseases.[2]

In this application note, initial scouting experiments for the development of a stability-indicating method for esomeprazole are reported. An automated method scouting workflow is presented, which is easy to perform and delivers faster method development. For this purpose, ChromSword Chromeleon Connect, which consists of the software modules Scout, Developer, ReportViewer, and AutoRobust, is combined with a Thermo Scientific™ Vanquish™ Flex UHPLC system, together with a Thermo Scientific™ ISQ™ EM mass detector. UV- and mass spectra-based peak tracking is used to evaluate separations for the screening of columns and mobile phases. The most promising column, mobile phase buffer, and buffer pH can be used as starting conditions for further gradient optimization using the Developer module of ChromSword Chromeleon Connect.

Experimental
Chemicals
- Deionized water, 18.2 MΩ/cm at 25 °C, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136149)

- Acetonitrile, Optima™ LC/MS grade, Fisher Chemical™ (P/N A955)

- Methanol, Optima™ LC/MS grade, Fisher Chemical™ (P/N A456-212)

- Formic acid, Optima™ LC/MS grade, Fisher Chemical™ (P/N A117)

- Ammonium formate, Optima™ LC/MS grade, Fisher Chemical™ (P/N A115)

- Ammonium bicarbonate, Fisher BioReagents, Fisher Chemical™ (P/N 10532775)

- Esomeprazole magnesium, Hydrochloric acid (HCl), fuming, 37%, Hydrogen peroxide (H_2O_2), 30%, Sodium hydroxide (NaOH), Ammonium hydroxide (NH_4OH), >25% (purchased from a reputable vendor)

Sample handling
- Fisherbrand™ Mini Centrifuge (P/N 12-006-901)

- Thermo Scientific™ Orion 3 Star™ pH Benchtop Meter (P/N 13-644-928)

- Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer (P/N 14-955-152)

- Thermo Scientific™ Finpipette™ F1 Variable Volume Single-Channel Pipettes: 100–1000 μL (P/N 4641100N), 10–100 μL (P/N 4641070N), 1–10 μL (P/N 4641030N)

- Vials (amber, 2 mL), Fisher Scientific™ (P/N 15508760)

- Snap Cap with Septum (Silicone/PTFE), Fisher Scientific™ (P/N 10547445)

Instrumentation

Thermo Scientific Vanquish Quaternary Flex system consisting of:

- Vanquish Flex System Base (P/N VF-S01-A)

- Vanquish Quaternary Pump F (P/N VF-P20-A)

- Vanquish Split Sampler FT (P/N VF-A10-A)

- Vanquish Column Compartment H (P/N VH-C10-A-02)

- Vanquish Diode Array Detector FG (P/N VF-D11-A)

- Thermo Scientific ISQ EM Single Quadrupole Mass Spectrometer (P/N ISQEM-ESI)

Sample preparation

The sample solution was prepared prior to injection by pooling three different stress degradation samples. 450 µL alkaline degradation sample, 450 µL oxidative degradation sample, and 100 µL acidic degradation sample were mixed. Forced degradation samples of esomeprazole under acidic, alkaline, and oxidative stresses were performed as follows:[2]

Acidic degradation

20 mg of esomeprazole was put into a 100 mL volumetric flask and 25 mL of 0.1 N HCl was then added. After 1 hour at room temperature, 0.1 N NaOH was added to neutralize the solution. Methanol was added to the flask to fill the sample up to 100 mL.

Alkaline degradation

20 mg of esomeprazole was put into a 100 mL volumetric flask and 20 mL of 1 N NaOH was then added. After 1 hour in a water bath at 80 °C, 1 N HCl was added to neutralize the solution. Methanol was added to the flask to fill the sample up to 100 mL.

Oxidative degradation

20 mg of esomeprazole was put into a 100 mL volumetric flask and 50 mL of 0.3% H_2O_2 was then added. After 3.5 hours at room temperature, methanol was added to the flask to fill the sample up to 100 mL. The degraded sample solution was then kept overnight at -20 °C to quench the reaction.

Mobile phase buffer preparation

For the purpose of method scouting, three buffer solutions were used as mobile phase A:

- 20 mM ammonium formate buffer at pH 3.0

- 20 mM ammonium bicarbonate at pH 7.0

- 20 mM ammonium bicarbonate at pH 8.0.

The pH was adjusted with formic acid or ammonium hydroxide.

Chromatographic conditions

Parameter	Value
Columns tested	Thermo Scientific™ Accucore™ C18 (100 × 2.1 mm, 2.6 µm) P/N 17126-102130 Thermo Scientific™ Hypersil GOLD™ C8 (100 × 2.1 mm, 3 µm) P/N 25209-102130 Thermo Scientific™ Acclaim™ Polar Advantage II (PA2) (150 × 2.1 mm, 2.2 µm) P/N 071401
Mobile phase buffers and pH tested	20 mM ammonium formate, pH 3.0 20 mM ammonium bicarbonate, pH 7.0 20 mM ammonium bicarbonate, pH 8.0
Mobile phase B	Acetonitrile
Gradient	Time (min) %B 0 15 9.3 25 13.9 35 15.9 35 16 15
Run time	30 min
Flow rate	0.6 mL/min
Column temperature	40 °C (still air)
Sampler temperature	4 °C
Injection volume	10 µL
UV detector parameters	Detection at 280 nm 3D scan 190–450 nm Data collection rate 10 Hz Response time 0.2

MS detector settings

Parameter	Value
Ionization mode	ESI
Polarity (Spray voltage)	Positive (+3,000 V)
Full scan (Basic mode)	*m/z* 100–450
Min. baseline peak width	6 s
Desired scans per peak	15
Acquisition rate	2.5 Hz
CID voltage	15 V
Vaporizer temperature	338 °C
Ion transfer tube temperature	300 °C
Gas flow pressures	Sheath gas: 56.9 psig Auxiliary gas: 6.5 psig Sweep gas: 0.5 psig

Chromatography Data System

The Scout module of ChromSword Chromeleon Connect, together with Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS), was used for data acquisition during method scouting. The ReportViewer module of ChromSword Chromeleon Connect was used for data analysis and evaluation.

The `Track and Rename peaks' feature in ReportViewer allows the automatic labeling of peaks on different chromatograms, based upon peaks from a selected reference chromatogram. In column selection (discussed in the Results and discussion), a separation on the Hypersil GOLD C8 column with an ammonium bicarbonate buffer of pH 8.0 was used as the reference chromatogram. In buffer screening, a separation on the Accucore C18 column with ammonium bicarbonate buffer of pH 8.0 was used as a reference chromatogram. Appropriate option and/or values for parameters (such as the option for `Spectra and area tracking', minimum spectra correlation coefficient, minimum area correlation coefficient, and UV-MS time difference) were applied. It is worth noting that the time delay (i.e., UV-MS time difference) between the UV- and mass detector needs to be entered manually.

Step 1: Method scouting
- ChromSword Chromeleon Connect software module: Scout
- Screen for a promising combination of column, solvent, and mobile buffer pH

Step 2: Data analysis
- ChromSword Chromeleon Connect software module: ReportViewer
- Process chromatograms, UV and mass spectra, generate reports

Step 3: Method optimization
- ChromSword Chromeleon Connect software module: Developer
- Fine tune method parameters such as gradient, flow rate, and temperature

Step 4: Method robustness testing
- ChromSword Chromeleon Connect software module: AutoRobust
- Verify the method is robust when changing separation parameters (such as pH, flow rate, and temperature)

Figure 1. General workflow for automated method development using ChromSword Chromeleon Connect

Results and discussion

Automated method scouting using ChromSword Chromeleon Connect

The Vanquish Method Development system, combined with ChromSword Chromeleon Connect, streamlines the entire method development process, providing automation of all method development tasks such as method scouting, method optimization, and method robustness testing. A schematic overview of the general workflow for automated method development using ChromSword Chromeleon Connect software is shown in Figure 1. First, key chromatographic parameters such as columns, solvents, and mobile phase buffer composition and pH are screened (step 1). Data obtained from method scouting were analyzed and evaluated using the ReportViewer module of ChromSword Chromeleon Connect (step 2). Data analysis in step 2 is also performed for data from method optimization (step 3) and from method

robustness testing (step 4). The detailed chromatographic parameters such as gradient, flow rate, and temperature are then further optimized with the column, solvent, and mobile phase buffer selected in step 1 (step 3). Finally, method robustness testing is performed to verify that the final method (developed in step 3) remains unaffected regardless of slight variations in parameters such as pH, flow rate, and temperature (step 4).

This application note focuses on step 1, the method scouting step. Three columns and three mobile phases were screened to develop a stability-indicating method for esomeprazole and its degradation products. For this purpose, the Scout module of ChromSword Chromeleon Connect was used with a Vanquish Method Development UHPLC System coupled to ISQ EM mass detector. A new project (or method) to be run was created within minutes, which includes a sequence with all candidate column-buffer combinations (see the chromatographic conditions in the experimental section for more details). A total of nine methods, consisting of three columns and three mobile phase buffer conditions were then automatically run in duplicate. Accordingly, a total of 18 injections of the same pooled forced degradation sample were performed in an unattended manner, taking around 9 hours (data acquisition time). The data acquisition time of 9 hours included run time and the time for column equilibration, purging, and final wash-out method. Data analysis and evaluation was then performed using the ReportViewer module of ChromSword Chromeleon Connect (step 2). The most promising column and mobile phase buffer pH were selected within approximately 1 hour (data analysis time). Specifically, six scouting runs using three columns were first evaluated for the column screening. The buffer screening was then followed with the selected column, where six scouting runs using three mobile buffers were evaluated.

Table 2 lists known degradation products (or impurities) of esomeprazole, found in Reference 2, and corresponding formula and masses. The pooled sample, consisting of

0.2 mg/mL esomeprazole and degradation products generated at different stress conditions, was injected for the scouting runs. The gradient profile in Reference 2 was used for the initial screening for columns and mobile phase conditions.

Chemical structures of esomeprazole and degradation products listed in Table 2 are shown in Figure 2.
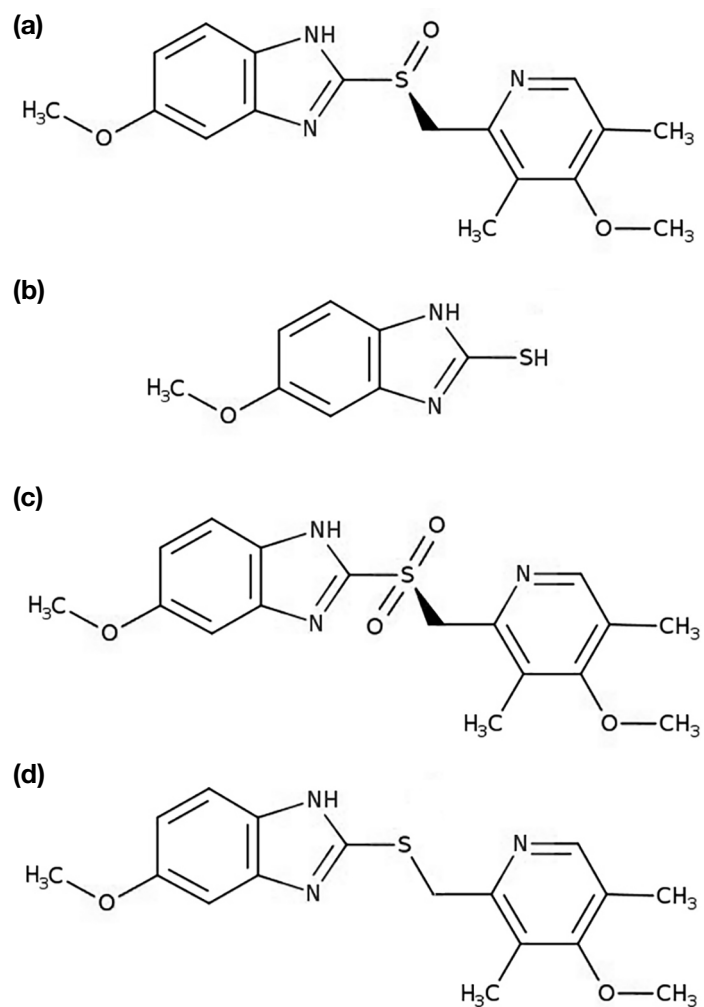

Figure 2. Chemical structures of (a) esomeprazole, (b) impurity 1, (c) impurity 3 (Imp-3), and (d) impurity 4 (Imp-4)

Name	Stress condition	Formula	Monoisotopic mass [Da]	Mass [M + H]+ [Da]
Esomeprazole	Not available	$C_{17}H_{19}N_3O_3S$	345.1	346.1
Impurity 1	Alkaline degradation	$C_8H_8N_2OS$	180.0	181.0
Impurity 3	Acidic degradation	$C_{17}H_{19}N_3O_2S$	329.1	330.1
Impurity 4	Oxidative degradation	$C_{17}H_{19}N_3O_4S$	361.1	362.1

Table 2. Formula and monoisotopic masses and SIM masses of esomeprazole and known impurities generated at different stress conditions.[2]. The protonated masses of these impurities were monitored during the method scouting.

Column screening and UV- and MS spectra-based peak tracking

For the purpose of screening, three columns (Hypersil GOLD C8, Accucore C18, Acclaim Polar Advantage II) were selected for evaluation. According to the U.S. Pharmacopeial convention (USP) website,[3] the F-values of the Acclaim Polar Advantage II column and the Accucore C18 column (compared to the Hypersil GOLD column) are 40.07 and 12.64, respectively. This implies that the columns have orthogonal selectivity since the F-values are much higher than 3. The column screening was performed by evaluating parameters related to the column selectivity and peak shape, namely total number of resolved peaks, resolution, peak asymmetry, and peak width. The ReportViewer allows the filtering of user-defined (or selected) results, which can then be exported in a generic format. Of the three columns, the Polar Advantage II (PA2) column was excluded from further screening because the fewest number of peaks were observed. Results of above-mentioned parameters for API and related degradation products on the other two columns are summarized in Table 3.

Names of compounds (i.e., API and related degradation products) assigned for peaks on the two columns were also summarized in Table 3. Mass spectra data, provided by the ISQ EM mass detector, complemented UV data to assign and confirm compounds. For example, peaks 7 and 9 on the Hypersil GOLD C8 column were identified as known impurities of esomeprazole (i.e., impurity-4 and impurity-3, respectively), simply by comparing the UV chromatogram of the pooled degradation sample with two extracted ion chromatograms (XICs), as shown in Figure 3. The XICs for impurity-3 and impurity-4 were obtained at m/z 330 and m/z 362, respectively. Impurity-1 was not found in the pooled sample. Apart from the early-eluting solvent related peaks (peaks 1–3), API (peak 8) and peaks due to the impurities 3 and 4 (peaks 9 and 7), seven additional unknown peaks (UK-1, UK-2, UK-3, UK-4, UK-5, UK-6, UK-7) were found in the sample. Peaks on the Accucore C18 column were then assigned by automatic labeling using the 'Track and Rename peaks' feature in data processing in ReportViewer (see the experimental section for parameter settings), based on those on the Hypersil GOLD C8 column. The analyses of UV spectra between peaks were performed to ascertain if peaks were labeled correctly. No change in peak elution order between the two columns was found.

As shown in Table 3, the Accucore C18 column yielded the greatest number of peaks resolved with R_s >1.5 and better peak shapes, and thus was selected as the most promising column. All peaks due to esomeprazole and degradation products on this column were baseline separated (R_s > 2.0).

Table 3. Resolution (R_s), asymmetry, and peak width at half-height of esomeprazole and related degradation products on Hypersil GOLD C8 and Accucore C18 columns, along with compound name assigned. 20 mM ammonium bicarbonate buffer pH of 8.0 and acetonitrile were used as solvents.

| Peak #	Compound name	Hypersil GOLD C8			Accucore C18		
		R_s	Asymmetry	Width (at 50% height) (min)	R_s	Asymmetry	Width (at 50% height) (min)
1		0.9	1.47	0.04	2.2	6.09	0.04
2		1.2	3.07	0.04	1.2	1.73	0.01
3		4.0	1.89	0.06	2.0	2.4	0.08
4	UK*-1	4.4	2.8	0.07	8.8	1.98	0.09
5	UK-2	1.1	2.29	0.47	2.1	1.02	0.16
6	UK-3	3.7	1.29	0.16	3.5	3.91	0.28
7	Imp-4	5.5	1.03	0.19	7.4	1.07	0.13
8	API	15.12	1.15	0.21	25.7	1.21	0.14
9	Imp-3	10.58	1.16	0.18	17.7	1.03	0.11
10	UK-4	1.4	1.19	0.13	3.3	1.01	0.08
11	UK-5	3.1	1.21	0.12	2.8	1.13	0.07
12	UK-6	0.9	1.15	0.11	2.0	1.26	0.06
13	UK-7	-	1.77	0.12	-	1.23	0.06

*UK denotes 'Unknown' impurity.
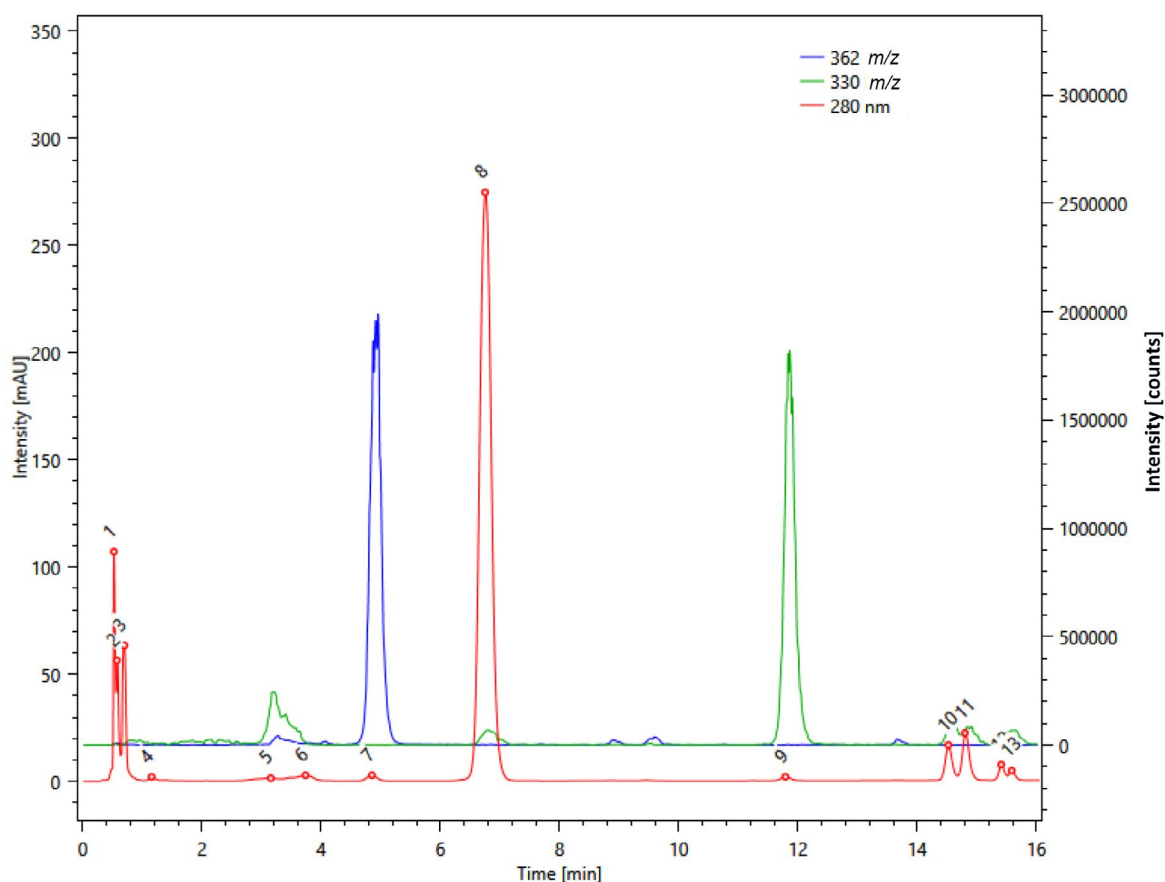
Figure 3. UV chromatogram (red) and extracted ion chromatograms (XICs, blue and green) of esomeprazole and related degradation products, confirming impurity-3 (green, *m/z* 330.4) and impurity-4 (blue, *m/z* 362.4). The separation was obtained on the Hypersil GOLD C8 column with ammonium bicarbonate buffer at pH 8.0 and acetonitrile. The wavelength of 280 nm was used for UV detection.

Buffer screening and MS spectra-based peak tracking

Six scouting runs using three mobile phase buffers with acidic, neutral, and alkaline pH on the Accucore C18 column were evaluated for buffer screening. Similar separations between ammonium bicarbonate of pH 7.0 and pH 8.0 were observed. A slightly better separation was seen with ammonium bicarbonate buffer at pH 8.0 (data not shown). Therefore, further tests were performed with ammonium formate pH 3.0 and ammonium bicarbonate pH 8 only, where peak tracking between the two chromatograms was carried out. The UV spectral characteristics of compounds can be substantially changed by varying the buffer pH and solvent. In addition, impurities are present in low quantities compared to the API, potentially resulting in an obscured UV absorption signal due to the dominant background mobile phase spectra.[1] Therefore, mass spectra of the impurities were used to track peaks in the two chromatograms. The ISQ EM mass detector facilitates accurate, straightforward, and rapid peak tracking, used together with ChromSword Chromeleon Connect.

By comparing with the corresponding total ion chromatogram (TIC) (Figure 4b), peaks 4, 5, 6 on the UV chromatogram in Figure 4a were confirmed as impurity-4 (*m/z* 362.1), API (*m/z* 346.1), and impurity-3 (*m/z* 330.1). Peaks 2, 7, and 8 (indicated as UK-2, UK-4, and UK-5 in Table 3), showed *m/z* values of 284.2, 296.2, and 296.1. Peaks 1, 3, 9, and 10 in the UV chromatogram were not shown in the TIC, since these impurities were not detectable under the applied conditions. Peaks on the UV chromatogram (Figure 4c) using ammonium formate buffer pH of 3.0 were then assigned using both the 'Track and Rename peaks' feature in ReportViewer and mass spectra for peaks in the TIC (Figure 4b) as a reference chromatogram. It is worth noting that the time delay between UV and mass detectors can vary depending on compounds, so that peak labeling needs to be carefully checked using the *m/z* values, following the automated peak labeling. For example, the unknown impurity-2 (i.e., UK-2) was not labeled in the UV chromatogram, most probably due to time delay between the UV and mass detectors.
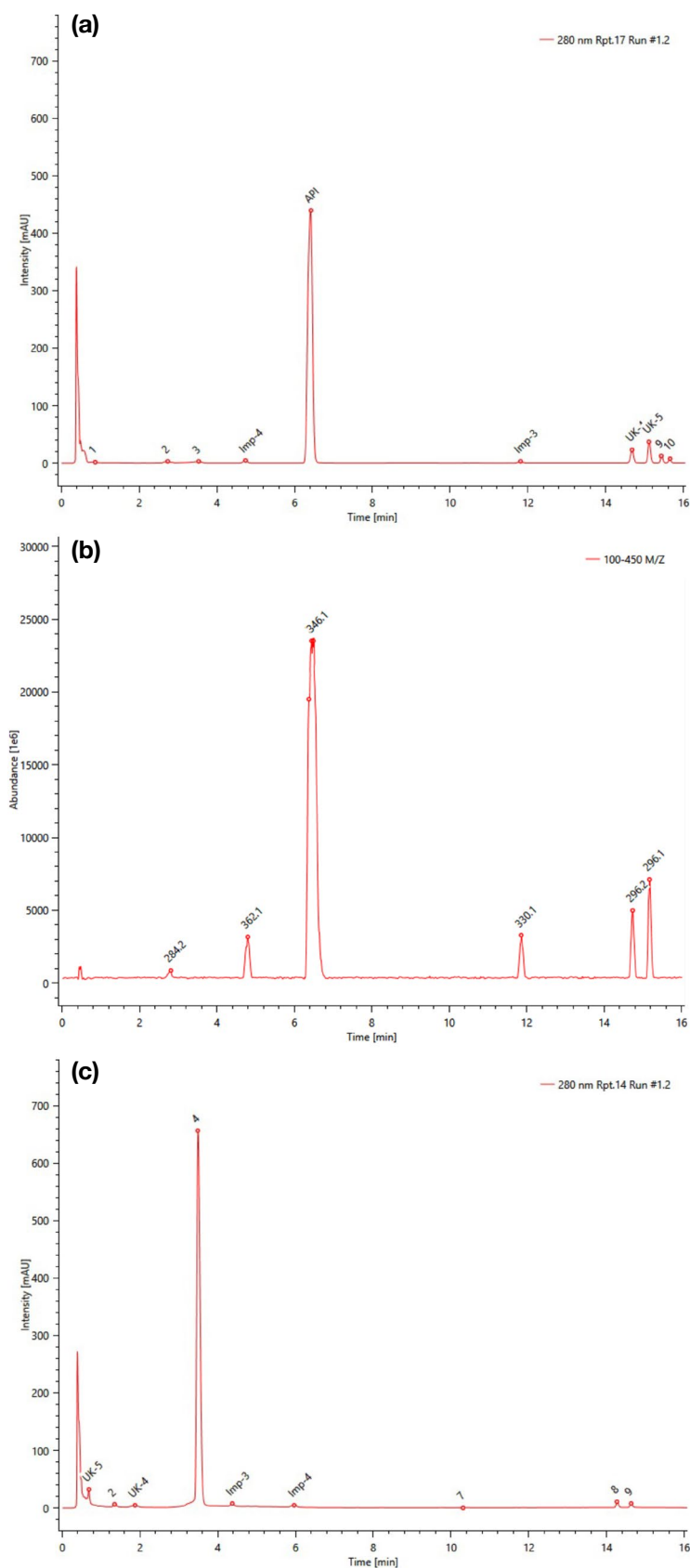
Figure 4. UV and TIC chromatograms using different mobile phase buffers for screening, showing changes of elution orders for impurity-4 and UK-4 between the two buffers. (a) UV at 280 nm using ammonium bicarbonate buffer at pH 8.0 and (b) TIC (*m/z* 100–450) using ammonium bicarbonate buffer at pH 8.0. Peaks in (b) are labeled with the most abundant ion detected in the mass spectrum of the peak. (c) UV at 280 nm using ammonium formate at pH 3.0.

Significant changes of elution order were observed between the two buffers. For example, impurity-4 eluted earlier than the API with the buffer of pH 8.0; whereas it eluted later than API with the buffer of pH 3.0. In addition, the UK-4 eluted later than the API with the buffer of pH 8.0; whereas it eluted earlier than API with the buffer of pH 3.0. As shown in Figure 4, the use of ammonium bicarbonate buffer at pH 8 produced more peaks and better resolution, resulting in selection of this mobile phase buffer as the best.

As a result, the Accucore C18 column and ammonium bicarbonate buffer of pH 8.0 were selected as a starting condition for further method optimization. The method optimization (i.e., fine-tuning of gradient profile and temperature) can be performed using the Developer module of ChromSword Chromeleon Connect.[4] The proposed systematic approach using ChromSword Chromeleon Connect software streamlines method development, reducing method development time and cost, as well as analysis time.

Conclusion

- The Scout module of ChromSword Chromeleon Connect, combined with the Vanquish Flex UHPLC system and ISQ EM mass detector, enabled unattended method screening for column and mobile phases.

- A proposed workflow resulted in rapid screening of the most suitable column and mobile phase buffer within 10 hours.

- By a systematic approach using method development software, the Accucore C18 column was rapidly selected as the most suitable column for separating esomeprazole and related degradation products.

- The combined use of UV and mass spectra facilitated accurate, straightforward, and rapid peak tracking during method scouting.

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