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IC-MS for the determination of organic acids in pharmaceutical solutions

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Goal

To develop a simplified ion chromatography (IC) application in conjunction with mass-selective detection to facilitate the identification and quantification of highly polar aliphatic and unsaturated organic acids in pharmaceutical solutions

Introduction

High-performance liquid chromatography (HPLC) and IC are used for a wide range of applications across the pharmaceutical industry. In drug discovery, for example, these methods are used to screen drug candidates, either as standalone tools or in conjunction with mass spectrometers. HPLC and IC workflows are also used in pre-clinical development to analyze *in vitro* and *in vivo* samples, and in clinical trials to gather data on drug safety and efficacy. These techniques also play an essential role in pharmaceutical manufacturing, including the collection of quality assurance/quality control (QA/QC) data and the validation of cleaning procedures.[1]

One of the many challenging tasks faced by pharmaceutical companies is the determination of

short-chained aliphatic and unsaturated organic acids during development and manufacture. This is necessary when assessing impurities in a starting material (educt), determining breakdown products in formulation stability monitoring, or controlling the cleaning of production lines. Because these short-chained, low molecular weight organic acids are hydrophilic and very polar in nature, their identification and quantification are complicated at trace levels.

There is a range of analytical techniques available to quantify organic acids. Ion pair chromatography, ion suppression chromatography, and anion exchange chromatography with weak anion-exchange columns are often used in combination with UV-detection.[2-4] However, these approaches do not offer sufficient sensitivity and selectivity for the trace-level detection

of short-chained organic acids in complex samples. Alternative approaches include the use of mass spectrometry (MS) in conjunction with reversed-phase ion suppression chromatography[4] and gas chromatography (GC), and capillary zone electrophoresis (CE) – although each presents its own unique sets of analytical challenges. For example, reversed-phase ion suppression chromatography does not provide sufficient chromatographic retention for the analysis of monobasic C1-C4 organic acids, while GC requires complex sample preparation such as continuous solid-phase extraction, liquid-liquid extraction, or derivatization.[5-7] Meanwhile, CE has been successfully used for the determination of low molecular weight aliphatic carboxylic acids,[8,9] but the low sensitivity of the detection methods used (UV or indirect UV), the easy overloading of the fused silica capillary, and the strong matrix dependency of the migration times have so far prevented the broader application of CE for determining trace-level concentrations of organic acids.

A more promising solution is to use anion-exchange chromatography at elevated pH with suppressed conductivity detection. This method offers high chromatographic selectivity and sufficient retention for short-chained organic acids, a higher detection sensitivity, and high compatibility with complex matrices.[2, 10-13] Using a continuously regenerated, membrane-based suppressor leads to lower background conductivity, allows gradient elution, and increases the sensitivity of conductivity detection due to the conversion of the analytes into their corresponding acids. MS detection augments this high chromatographic sensitivity and selectivity, where the suppressor acts as a continuously regenerated desalter facilitating the combination of high-pH eluents with MS-detection.

This application note illustrates the use of an easy-to-implement IC-MS method for the determination of aliphatic and unsaturated organic acids in pharmaceutical solutions. This workflow employs both suppressed conductivity and MS detection to increase the information available from each sample. Samples were separated on a high-resolution Thermo Scientific™ Dionex™ IonPac™ AS11-HC-4µm column set using a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system with suppressed conductivity detection. The sequential MS detection was achieved using a Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer. The ISQ EC single quadrupole mass spectrometer increases analytical confidence by providing selectivity, sensitivity, and confirmation of analyte identity.[14] This mass spectrometer can run in Full Scan and Single Ion Monitoring (SIM) modes, giving the flexibility to either scan a mass range for all detectable analytes or focus on a specific compound.

The study presented here demonstrates that co-eluting organic acids can be accurately quantified with mass spectrometric detection. Analyte identity can also be confirmed with high levels of accuracy, especially for low molecular weight organic acids such as formate, which has a mass-to-charge (*m/z*) ratio of 45. The experiments described below were completed in collaboration with a leading pharmaceutical company. We used 25 mg/L 2-butynoic acid as an example of a pharmaceutical solution.

Experimental

Equipment and consumables

- Dionex Integrion HPIC system including:
  - Eluent generator
  - Pump
  - Degasser
  - Conductivity detector (CD)
  - Column oven temperature control
  - Detector compartment with temperature control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with 250 µL sample syringe (P/N 074306) and 1.2 mL buffer line (P/N 074989)
- ISQ EC single quadrupole mass spectrometer (P/N ISQEC000IC)
- 2 Thermo Scientific™ Dionex™ AXP Metering Pumps (P/N 063973)
- Peak™ Scientific Genius NM32LA Nitrogen Generator (P/N 10-6022 (230 V))
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)

- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (P/N 088667)
- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fittings Kit (P/N 088798)
- Dionex AS-AP Autosampler Vials: 1.5 mL polypropylene vials with caps and septa (P/N 079812)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) version 7.2 SR9

Conditions

IC conditions	
IC system	Dionex Integrion HPIC system
MS detector	ISQ EC single quadrupole mass spectrometer
Columns	Dionex IonPac AG11-HC-4µm Guard, 2 × 50 mm (P/N 078036) Dionex IonPac AS11-HC-4µm Analytical, 2 × 250 mm (P/N 078035)
Eluent source	Dionex EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Dionex CR-ATC 600 (P/N 088662)
Gradient	Inject (0 min) 1 mM KOH (-5.0–8.5 min) 1–15 mM KOH (8.5–18.5 min) 15–30 mM KOH (18.5–28.5 min) 30–54 mM KOH (28.5–30 min) 1 mM KOH (31.5 min)
Flow rate	0.38 mL/min
Injection volume	25 µL in Push-Full mode
Temperature	40 °C (column compartment) 35 °C (detector compartment)
Backpressure	~3300 psi (100 psi = 0.6894 MPa)
Suppressed conductivity detection	Dionex ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) (P/N 088667) AutoSuppression in the dynamic regeneration mode (4 V), external water mode via Dionex AXP Pump, external water flow rate (0.4 mL/min)
Make-up solvent flow rate	0.1 mL/min
Background conductance	<0.5 µS/cm
Run time	36.5 min

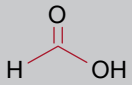
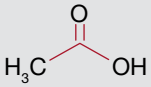
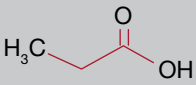
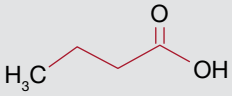
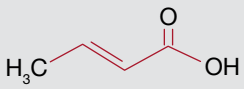
Mass spectrometric detection	
Ionization interface	Electrospray ionization (ESI), negative mode
Gas control	Sheath gas pressure: 50 psi Aux gas pressure: 5 psi Sweep gas pressure: 0.0 psi
Source voltage	-2500 V
Vaporizer temperature	450 °C
Ion transfer tube temperature	200 °C
SIM scan	Table 1
Full scan	Mass range: 40–250 *m/z*
Source CID voltage	5 V
SIM width	0.1 amu

Preparation of solutions and reagents

Deionized water with a resistivity of 18 MΩ·cm or better and a total organic carbon content (TOC) of less than 10 µg/L was used for eluent and standard preparation, and for diluting samples. Standards of organic acids of the highest available grade were obtained from Sigma-Aldrich Ltd. (Gillingham, United Kingdom). HPLC grade ammonia (0.25 M) and ammonium acetate (0.25 M) were sourced from Fisher Scientific (Loughborough, UK). The samples of 2-butynoic acid were provided by chemical and pharmaceutical companies from the US and the UK; 2-butynoic acid was chosen as the main matrix component based on their request.

Individual stock standard solutions were prepared at 10 mg/L or 50 mg/L (2-butynoic, crotonoic, and 2-pentynoic acid). Mixed working stock solutions of 1 mg/L and 100 µg/L resulted from the appropriate dilution of the respective stock solutions. From the 1 mg/L working solution, 500 µg/L, 200 µg/L, and 100 µg/L standards were prepared, and from the 100 µg/L working solution, 50 µg/L, 10 µg/L, 5 µg/L, and 1 µg/L standards were prepared.

Table 1. Analytes of interest, structures, molecular weight, and SIM conditions for MS-detection

Component	MW (g/Mol)	Quantitation ion (*m/z*)	CAS No.	Structure
Formic acid	46.025	45	64-18-6	
Acetic acid	60.05	59	64-19-7	
Propanoic acid "propionic acid"	74.079	73	79-09-4	
Butanoic acid	88.106	87	107-92-6	
Pentanoic acid	102.13	101	109-52-4	
Crotonic acid ((E)-But-2-enoic acid)*	86.09	85	3724-65-0	
2-Propiolic acid (Prop-2-ynoic acid)	70.05	69	471-25-0	
2-Butynoic acid (But-2-ynoic acid)*	84.07	83	590-93-2	
2-Pentynoic acid (Pent-2-ynoic acid)*	98.1	97	5963-77-9	

* IUPAC name

Results and discussion

Optimization of the make-up solvent

In applications combining a Thermo Scientific™ Dionex™ Reagent-Free™ IC instrument (RFIC™) with an ISQ EC single quadrupole mass spectrometer, the heated electrospray ionization (HESI-II) interface is typically used. The HESI-II probe allows the use of high temperatures and voltage to deliver better desolvation and enhanced sensitivity.

A protocol described by Wang et al. in 2009,[15] as well as another method recently published in 2019,[16] suggests the use of a make-up solvent containing acetonitrile in conjunction with a HESI-interface. As a result, solvent mixtures based on acetonitrile and water (volume fraction ϕ= 50%) were tested with and without ammonium acetate (12.5 mg/L) or ammonium hydroxide (25 mg/L) present. A make-up solvent containing ammonium acetate led to a significant loss of sensitivity (up to 65%) compared to the pure acetonitrile/water mixture. However, the ammonium hydroxide additive increased sensitivity for organic acids. Compared to pure acetonitrile/water, we found values between 20% and 200% higher for the different analytes tested. As a result, a make-up solvent consisting of acetonitrile/water plus 25 mg/L ammonium hydroxide was used throughout the experiments described below. Acetonitrile hydrolyzes to ammonia and acetate when left exposed to high-pH solutions; therefore, prepare the make-up solvent fresh daily.

Calibration experiments

Figure 1 shows an overlay of the chromatograms obtained with suppressed conductivity detection and MS detection using the conditions specified previously. With MS detection, all analytes can be determined free of interference, while in the suppressed conductivity trace, 2-butynoic acid and 2-propiolic acid co-eluted. Due to the overlap of 2-butynoic acid and 2-propiolic acid, only the MS data were processed.

For both detection methods, a second-order fit best characterized the calibration data. Following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidance[17] on the validation of analytical procedures in case of a higher-order fit, the analytical response should be described by an appropriate function of the concentration of an analyte in the sample. Hence, the evaluation of the analytical data was performed following the statistical approach of ISO 8466:2.[18]

Table 2 lists calibration data, as well as limits of detection (LOD) and limits of quantification (LOQ) for suppressed conductivity detection and MS-detection (using SIM).

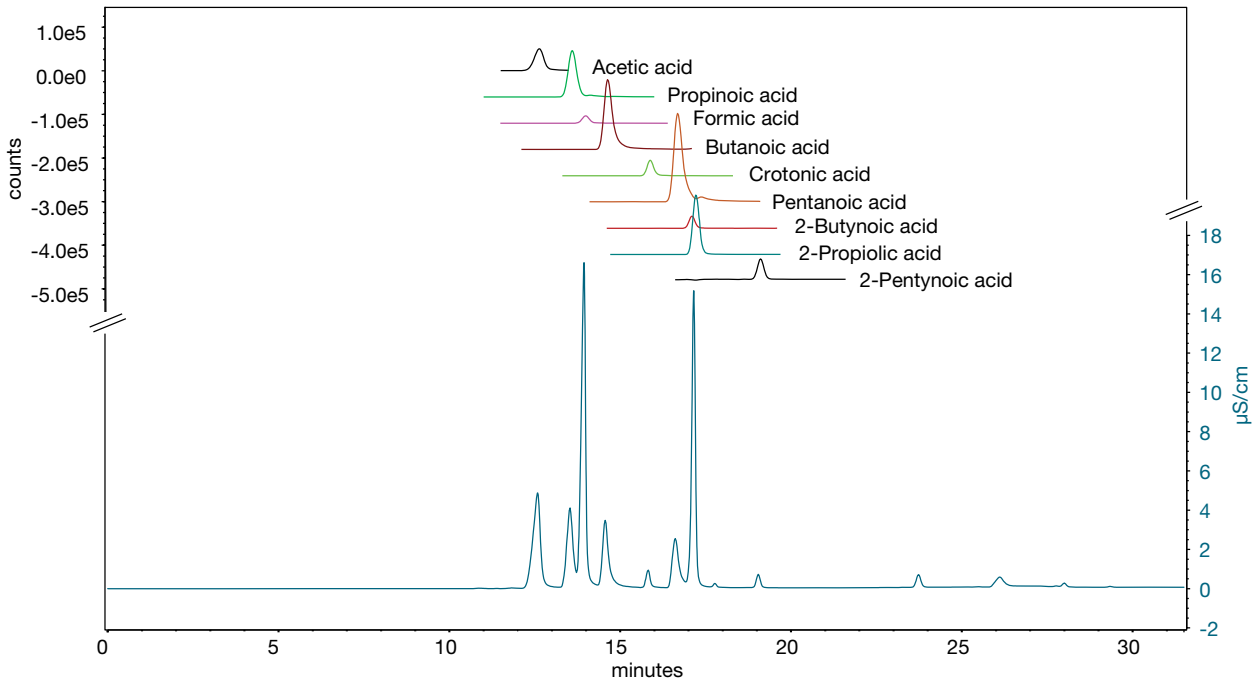


Figure 1. Conductivity (dark blue) and SIM chromatograms of all analytes (500 µg/L). Note that the signals are offset.

Table 2. Calibration data for MS-detection and suppressed conductivity

Compound	MS detection / SIM				Suppressed conductivity			
	Coefficient of determination	RSD	LOD (µg/L)	LOQ (µg/L)	Coefficient of determination	RSD	LOD (µg/L)	LOQ (µg/L)
Acetic acid	0.9997	3.00	5.8	18.9	0.99998	0.68	1.5	4.9
Propionic acid	0.9998	2.53	4.6	15.0	0.99997	0.88	1.9	6.2
Formic acid	0.9997	2.66	4.6	15.0	1.00000	0.32	0.8	2.4
Butanoic acid	0.9998	2.12	4.2	13.8	0.99997	0.98	2.2	7.1
Crotonic acid	0.9995	4.20	8.9	28.8	0.99995	1.31	3.3	10.6
Pentanoic acid	1.0000	0.63	1.3	4.2	0.99986	1.96	4.4	14.2
2-Butynoic acid	1.0000	1.04	2.0	6.6	-*	-	-	-
2-Propiolic acid	0.9994	3.80	6.4	21.0	-*	-	-	-
2-Pentynoic acid	0.9992	5.29	11.4	36.9	0.99999	0.76	2.0	6.4

Second-order calibration calculation; according to ISO 8466-2:2001,[18] probability 95%, n = 8. Calibrated range 1 µg/L to 500 µg/L for each analyte.
* Due to co-elution in suppressed conductivity detection, evaluation is not possible.

Both detectors showed LODs in the single-digit µg/L range and LOQs in the single- to double-digit µg/L range. Suppressed conductivity gave a better correlation of the data with the chosen calibration model, while the MS channels allowed for interference-free detection and quantification of all analytes across the explored concentration range.

Repeatability of MS-response for organic acids

Analytical methods used for impurity monitoring must be able to achieve accurate quantification when one organic acid is in significant excess compared to the others. To investigate the reproducibility of the response in this situation, an organic acid mix was prepared with a concentration of 2-butynoic acid at 25 mg/L and all the other organic acids at a level of 25 µg/L (or 0.1% of 2-butynoic acid) (Figure 2). The matrix-free solution consisted of all the organic acids at 50 µg/L (Figure 3).

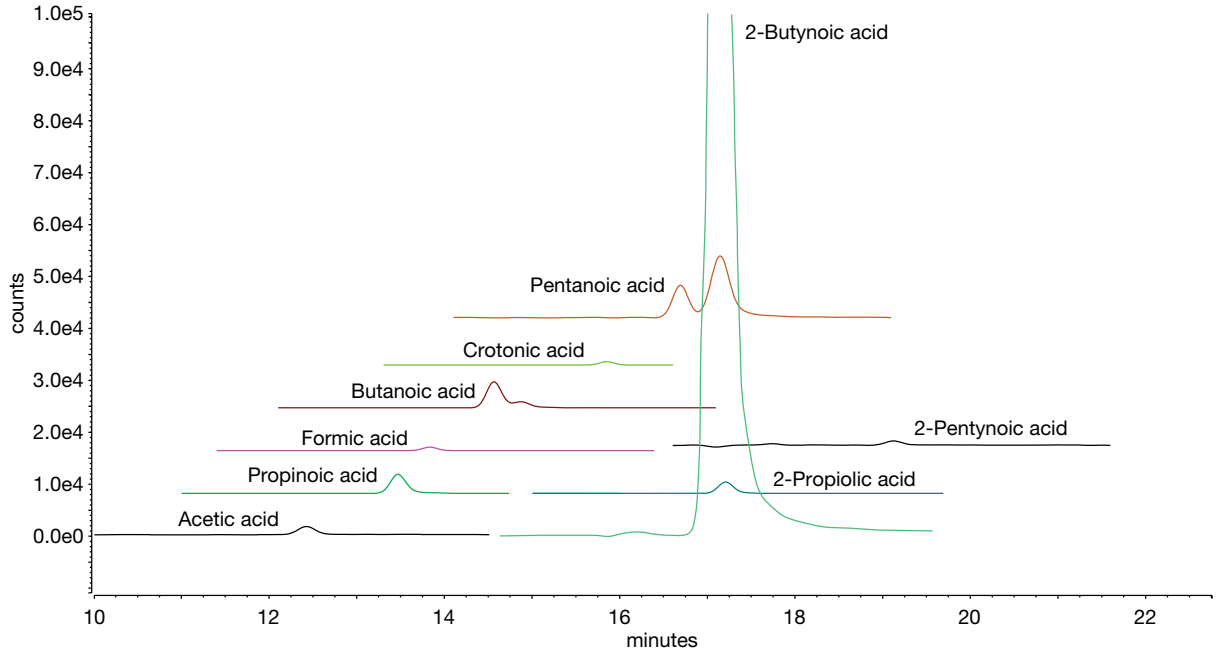


Figure 2. SIM chromatograms of organic acids (25 µg/L) in the presence of 25 mg/L 2-butynoic acid. Note that the signals are offset.

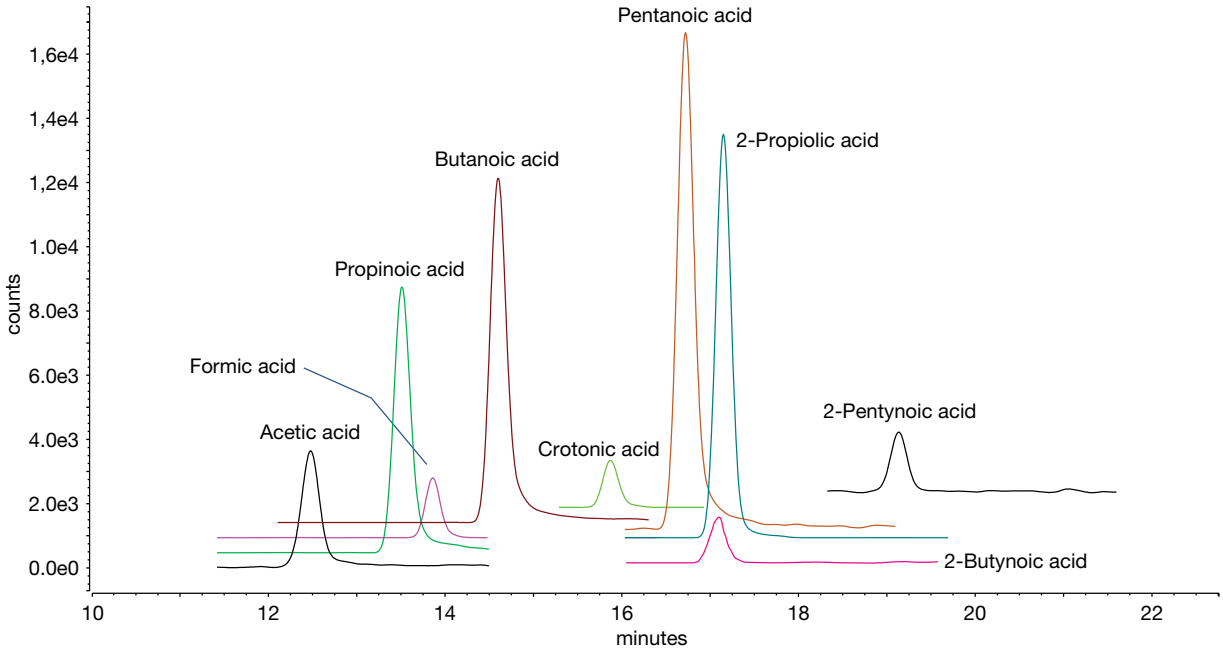


Figure 3. SIM chromatograms of the matrix-free organic acid solution (50 µg/L). Note that the signals are offset.

Both solutions were injected ten times, and the analytical results obtained for the SIM channels were compared (Table 3). The high concentration of 2-butynoic acid had no effect on the retention times or the reproducibility of MS-detection. Evaluation of the SIM channels showed comparable peak area and peak height RSDs in the range of 4–10% for the two samples, illustrating the selectivity and repeatability of IC-MS for trace analysis across all analyzed components, even in the presence of a high concentration of 2-butynoic acid.

The influence of a large excess of 2-butynoic acid on the response of lower concentrations of organic acids was tested in a similar manner. Working solutions containing 25 mg/L 2-butynoic acid with different concentrations of organic acids were analyzed accordingly. The concentrations of the organic acids added were 1.5 µg/L, 2.5 µg/L, 5 µg/L, 12.5 µg/L, 18.75 µg/L, and 25 µg/L, or 0.005%, 0.01%, 0.02%, 0.05%, 0.075%, and 0.1% of the 25 mg/L 2-butynoic acid. Even for concentrations below the calculated LOD (Table 4), propinoic acid, formic acid, butanoic acid, and 2-propiloic acid showed a response within the range of ±20%. Acetic acid, pentanoic acid, and crotonic acid showed a larger deviation from their respective average response factors below an added concentration of 5 µg/L (0.02%), while the 2-pentynoic acid response deviated below an added concentration of 12.5 µg/L (Figure 4).

Table 3. Robustness data for IC-MS organic acids in matrix-free and 2-butynoic acid matrix (n = 10)

	25 µg/L organic acids in 25 mg/L 2-butynoic acid matrix				Matrix-free standard at 50 µg/L			
		RSD				RSD		
Compound name	Ret. time (min)	Ret. time	Area	Height	Ret. time (min)	Ret. time	Area	Height
Acetic acid	12.4	0.08	5.7	6.7	12.5	0.06	7.9	7.7
Propinoic acid	13.5	0.07	5.7	6.2	13.5	0.05	4.1	4.0
Formic acid	13.8	0.05	6.2	6.2	13.9	0.04	9.0	8.1
Butanoic acid	14.6	0.07	4.5	4.6	14.6	0.05	5.3	5.4
Crotonic acid	15.9	0.04	5.5	5.5	15.9	0.03	4.5	5.6
Pentanoic acid	16.7	0.05	5.8	6.2	16.7	0.02	5.8	6.7
2-Butynoic acid	17.2	0.04	4.0	3.8	17.1	0.02	6.7	5.7
2-Propiolic acid	17.2	0.02	5.0	5.4	17.1	0.02	4.2	4.3
2-Pentynoic acid	19.1	0.03	9.5	6.9	19.1	0.01	5.1	5.3

Table 4. Response factors (area/concentration) versus the added level of organic acids

	Response factor					
Compound	1.25 µg/L (0.005%) added	2.5 µg/L (0.01%) added	5 µg/L (0.02%) added	12.5 µg/L (0.05%) added	18.75 µg/L (0.075%) added	25 µg/L (0.1%) added
Acetic acid	48	32	23	17	16	18
Propinoic acid	37	38	35	37	32	33
Formic acid	6	7	7	6	5	6
Butanoic acid	48	46	48	44	46	48
Crotonic acid	2	4	5	6	6	6
Pentanoic acid	37	39	66	57	58	57
2-Butynoic acid	25 mg/L matrix component					
2-Propiolic acid	18	19	21	19	19	21
2-Pentynoic acid	n.d.	12	11	7	7	7

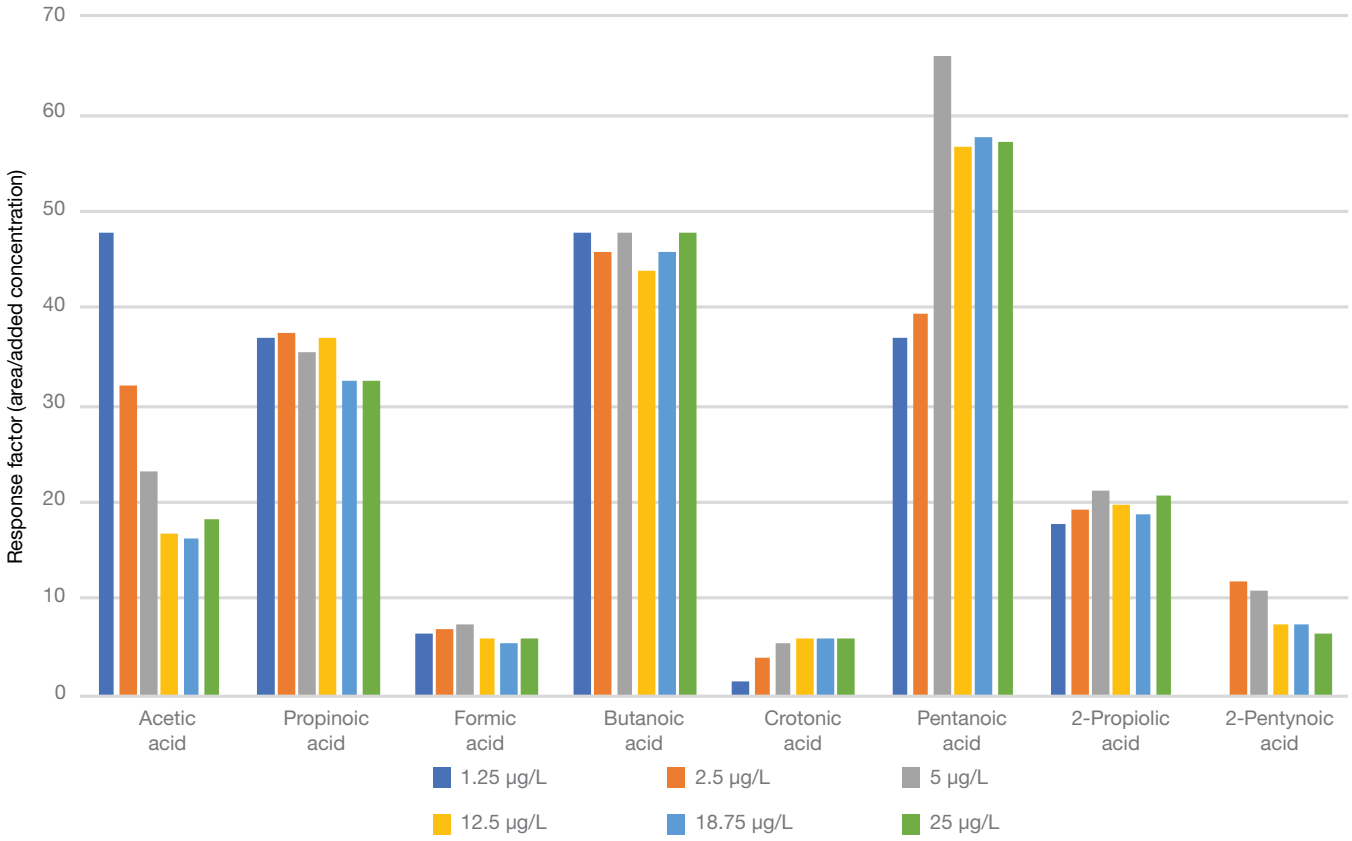


Figure 4. Response factors (area/concentration) versus the added level of organic acids

Conclusion

This study illustrates the use of a simplified IC-MS method to facilitate the trace amount determination of highly polar, low molecular weight aliphatic and unsaturated organic acids in pharmaceutical solutions. By improving the identification and quantification of these organic acids, pharmaceutical companies can overcome significant challenges in impurity monitoring.

In this workflow, the Dionex IonPac AS11-HC-4µm column supports a highly selective separation of the analytes in question. The high capacity of the column ensures the matrix tolerance needed to determine low concentrations of organic acids, i.e. below 0.1% relative to the chosen 2-butynoic acid matrix. The elevated column temperature (40 °C) improves chromatographic peak efficiencies and prevents the use of an organic solvent in the mobile phase. The use of continuously regenerated membrane-based suppressors allows the hyphenation of IC with MS, further adding to the analytical selectivity and sensitivity.

The combination with RFIC, i.e., the automatic eluent generation and dynamic electrolytic regeneration of the suppressor, results in a highly automated, highly reproducible instrument setup that can be easily validated. If desired, the instrument configuration can be extended with integrated monitoring of consumables, so that the analytical instrument configuration, the consumables in use, and their performance can be documented electronically.

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