INVESTIGATION OF THE RETENTION PROPERTIES OF ACTIVE PHARMACEUTICAL INGREDIENTS AND CONTAMINANTS IN HIGH PERFORMANCE ION CHROMATOGRAPHY SYSTEM

PhD thesis

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Introduction and aims

The aim of my thesis is to demonstrate the retention behaviour of contaminants in different active pharmaceutical ingredient (API) in ion chromatography. My objective was to highlight the role of ion chromatography, and in particular ion exchange chromatography, in the analysis of APIs/formulations by means of methods developed for the determination of different ions. My dissertation also focuses on the solubility by ion chromatography of compounds whose determination would be difficult and/or only feasible with mass spectrometer.

The first part of my thesis gives a brief literature review of the basics of chromatography and the correlations I used in my experiments. In this part I briefly and concisely describe ion exchange chromatography and the parts of the instrument used for the measurements. In the next I group the compounds that can be measured by ion-exchange chromatography according to their determination.

My results are presented in the first determination of acetic acid by gas chromatography and ion chromatography. I compared my developed methods through a limit level validation to determine which technique is advantageous for the determination of acetic acid from an API.

My task was to determine sodium azide, when is used under the API production in the synthesis pathway to form the tetrazole ring . To meet the stringent criteria set by the authorities, I present the determination of sodium-azid in cilostazol API and the development of the method, which includes testing the efficiency of the method and the retention behaviour of the azide ion under the influence of temperature variation. Finally, the developed method is validated at limit level according to the relevant international guidelines.

I have further investigated the retention behaviour of tris, sodium and lithium cations in a highly matrix-loaded system and demonstrated that this system is suitable for improving the resolution between tris and sodium ions.

Finally, I have dealt with the determination of *N*-bromosuccinimide in prasugrel API. I have applied the developed sample preparation step at a completely different structure (favipiravir) from prasugrel too to demonstrate that the sample preparation, or some elements of it (alkaline solvent, extraction), can be applied in general to the formation of bromide from *N*-bromosuccinimide. The developed method was validated at limit level for the prasugrel API.

Experiments and applied methods

The ion chromatography instrument used in my measurements is a Dionex ICS 5000 ion chromatograph (Thermo Scientific, Waltham, MA, USA). This instrument is a dual-channel instrument, so it is capable of measuring both anions and cations, even simultaneously. The ion chromatography system is equipped with an eluent generator with potassium hydroxide (KOH cartridge) on the anionic side and methanesulfonic acid (MSA cartridge) on the cationic side. Each side is equipped with a conductivity detector. The system also includes an UV detector in series after the conductivity detector of the respective side. The units of the ion chromatograph are thermostable. For the injection, I used sample injection loops of 10 μL and 100 μL for the anionic side and 100 μL for the cationic side. The suppressor is a dynamic electrochemically regenerated membrane cell, which is operated by recirculation or external regeneration as required. The external regeneration was solved by the cationic side pump system with purified water. Purified water (18.2 MΩcm) was used for the measurements and the eluents was supplied by the Elga PureLab Option water purification system. All the chemicals used for my tests were of analytical grade purity. Instrument control, data recording and chromatogram evaluation were performed with Chromeleon 7. Dionex Version 7.1.3.2425 (Thermo Scientific,Waltham, MA, USA). Gas chromatography measurements were performed using a Perkin-Elmer Autosystem gas chromatograph and a Perkin-Elmer HS-40 automated (PerkinElmer, Inc., Waltham, USA) headspace sampler and flame ionization (FID) detector. MarvinSketch 14.9.8.0 (Chemaxon, Budapest, Hungary) software was used for ionization analysis of compounds. Separations were performed using Dionex (Thermo Scientific, Waltham, MA, USA) chromatography columns. For anionic studies, a high capacity AS11-HC column (2x250 mm, d_p = 13 μm) with a corresponding pre-column (AG11-HC, 2x50 mm, d_p = 13 μm) and AS23 column (2x250mm, d_p = 13 μm) with corresponding feed column (AG23, 2x50mm, d_p = 13 μm). Both types of stationary phase alkanol with quaternary ammonium groups were functionalized latex agglomerated anion exchange columns. For the cationic assays, I used a CS16 (3x250 mm) analytical column and the corresponding preheater column (3x50 mm). The cation exchange stationary phase in this case is a carboxylic functional group containing surface.

For the gas chromatographic analyses, I used a DB-FFAP (30 m/0.32 mm/0.25 μm) (nitroterephalic acid-polyethylene glycol) type stationary phase.

New scientific results: thesis points

My achievements can be summarised as follows.

1. Ion chromatography is preferable to gas chromatography using a flame ionisation detector (FID) for the direct determination of acetic acid in an active pharmaceutical ingredient (API). The ion chromatography technique allows the determination of acetic acid contamination in the API with a lower limit of detection from less sample amount. Depending on the applied ion chromatographic analytical parameters, significantly better relative standard deviation values (five times lower for system precision and one and a half times lower for repeatability) can be achieved, and the analysis time can be reduced by at least 30% with 10% better recovery from 50 times less API.

2. The amount of sodium azide used in the manufacture of the cilostazol API can be determined at a concentration of 7,50 ppm by ion chromatography using liquid-liquid extraction sample preparation. The difference in enthalpy changes determined during the ion exchange processes between the azide ion and the hydroxide ions of the eluent is negligible (<1kJ/mol), resulting in a temperature-independent retention behavior of the azide ion. The results of the method, validated at limit level according to international standards, show that the analysing method is specific, robust, and precise and accurate at limit level.

3. In case of cation-exchange ion chromatography, matrix effects can be taken into account in method development for high-capacity weak cation exchangers to ensure adequate resolution of organic and inorganic cations that do not separate before the matrix component. On a given stationary phase (IonPac CS16), baseline separation of organic (tris) and inorganic (sodium) cations coeluting before the ammonium cation is achieved by using a 1.25% ammonium hydroxide matrix at room temperature (25°C). The increase in resolution between the two peaks results from a complex mechanism of matrix effects of ammonium hydroxide, which includes alkalinization of the eluent, buffer capacity of the matrix, change in the ionization state of the tris-cation, self-elution, and increase in the cation exchange capacity of the column used at high pH.

4. The low limit of detection of *N*-bromosuccinimide (NBS) in two different APIs (prasugrel, favipiravir) can be successfully achieved by ion chromatography through the analysis of bromide ions released from NBS during hydrolysis in the presence of sodium thiosulfate. For the Prasugrel API, the method can be reliably applied at the 25 ppm level after liquid-liquid extraction, while for favipiravir API the maximum allowable concentration of 37,5 ppm of NBS can be accurately measured by ethyl acetate extraction. For the Prasugrel, validation of the method demonstrated its specificity, accuracy, precision and linearity over the concentration range investigated.

Scientific publications, lectures and posters

Publications serving as the basis of thesis points

1. B. Páll, Zs. Gyenge, R. Kormány, K. Horváth Determination of Genotoxic Azide Impurity in Cilostazol API by Ion Chromatography with Matrix Elimination. Separations. 2021; 8(10):162. https://doi.org/10.3390/separations8100162

2. B. Páll, I. Kapui, R. Kormány, K. Horváth. Development of Analytical Methods for the Determination of N-Bromosuccinimide in Different Active Pharmaceutical Ingredients by High-Performance Ion Chromatography with Suppressed Conductivity Detection. Separations. 2023; 10(1):15. https://doi.org/10.3390/separations10010015

3. Páll, B., Kormány, R., Horváth, K. Az ionkromatográfia alkalmazhatóságának lehetőségei a gyógyszeranalitikában [Potential applications of ion chromatography in drug analysis]. Scientia et Securitas 3, 3, 227-233, Available From: AKJournals https://doi.org/10.1556/112.2022.00110

4. B. Páll, R. Kormány, K. Horváth Utilization of Matrix Effect for Enhancing Resolution in Cation Exchange Chromatography. Molecules. 2024; 29(15):3637. https://doi.org/10.3390/molecules29153637

Presentation based to the topic of the dissertation

International conferences

1. **B. Páll**, I. Kapui, R. Kormány, K. Horváth:Development of an Analytical Method for the Determination of N-bromosuccinimide in Two Different Active Pharmaceutical Ingredients by High-performance Ion Chromatography with Suppressed Conductivity Detection, *33rd International Symposium on Chromatography – ISC 2022*, 2022. szeptember.18-22., Budapest, Hungary. https://isc2022.hu/programme/

2. **B. Páll**, R. Kormány, K. Horváth :Matrix effects in cation exchange chromatography, *13th Balaton Symposium*, 2023.09.04-06., Siófok, Hungary. https://2023.balatonsymposium.hu/

3. **B. Páll**, Zs. Gyenge, R. Kormány, K. Horváth:Determination of Genotoxic Azide Impurity in Cilostazol API by Ion Chromatography with Matrix Elimination , *CONGRESSUS*

PHARMACEUTICUS HUNGARICUS XVII. AND EUFEPS ANNUAL MEETING 2024, 2024.05.23-25., Debrecen, Hungary. https://clubservice-event.hu/pdf-egyeb/abstracts_CPH2024.pdf

National conferences

1. **Páll B.**, Gyenge Zs., Kormány R., Horváth K.: Genotoxikus azid szennyezés meghatározása cilostazol hatóanyagból mátrix eliminációval, *Műszaki Kémiai Nap 2021 online konferencia*, 2021. április 21., Veszprém. https://mkn.mik.uni-pannon.hu/index.php/hu/program-2021.html

2. **Páll B.**, Gyenge Zs., Kormány R., Horváth K.: Genotoxikus azid szennyezés meghatározása cilostazol hatóanyagból mátrix eliminációval, *METT25 jubileumi konferencia*, 2021.10.18-20, Egerszalók. https://mett25.hu/tudomanyos-program/

3. **Páll B.**, Kapui I., Kormány R., Horváth K.: Gyógyszerhatóanyagban található *N*-brómszukcinimid szennyezés meghatározása nagyhatékonyságú ionkromatográfiás módszerrel, *Műszaki Kémiai Napok 2023*, 2023. április 18-20., Veszprém. https://mett25.hu/tudomanyos-program/

4. **Páll B.**, Kormány R., Horváth K.: Gyógyszerhatóanyagban található ecetsav szennyezés gáz- és ionkromatográfiás meghatározásának összehasonlítása, *XLVI. Gyógyszeranalitikai Továbbképző Kollokvium*, 2023.április 20-22., Nyíregyháza. https://mgyt.hu/wp-content/uploads/2023/03/Nyi%CC%81regyhaza-program-2023-03-13.pdf

5. **Páll B.**,Kormány R., Horváth K.: Mátrixhatás kationcserés ionkromatográfiában, *Műszaki Kémiai Napok 2024*, 2024.04.16-18., Veszprém. https://mk.uni-pannon.hu/images/Dokumentumok/MKN2024_Book_of_Abstracts.pdf

6. **Páll B.**, Kormány R., Horváth K.: Az ionkromatográfia alkalmazási lehetőségei a gyógyszeranalitikában, *Elválasztásitudományi Vándorgyűlés 2024*, 2024. november 7-9., Visegrád. https://2024.mettvandorgyules.hu/program/

Conference presentations related to the topic of the dissertation

International conference

1. **B. Páll**, R. Kormány, K. Horváth.:The Role of Sample Preparation Techniques in Liquid Chromatography Analysis of Pollutants of Active Pharmaceutical Ingredients, *33rd*

International Symposium on Chromatography – ISC 2022, 2022. szeptember.18-22., Budapest, Hungary https://isc2022.hu/programme/

International conference

1. **Páll B.**, Kormány R., Horváth K.:A mintaelőkészítés szerepe a gyógyszerhatóanyagok folyadékkromatográfiás vizsgálatában, *XLV. Gyógyszeranalitikai Továbbképző Kollokvium*, 2022. április 28., Kecskemét. https://mgyt.hu/wp-content/uploads/2022/04/Analitika-program-2022.pdf