Development of Fourier transform infrared spectrophotometric method for identification and determination of marketed metamizole tablet preparation

NERDY NERDY1*, EFFENDY DE LUX PUTRA2, NILSYA FEBRIKA ZEBUA3, CHRISTICA ILSANNA SURBAKI1, JIHAN SAFIRA2

1Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Institut Kesehatan Deli Husada Deli Tua, Deli Tua Timur, Deli Tua, Deli Serdang, Sumatera Utara, Indonesia, 20355
2Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Padang Bulan, Medan Baru, Medan, Sumatera Utara, Indonesia, 20222
3Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Tjut Nyak Dhien, Sei Sikambing, Medan Helvetia, Medan, Sumatera Utara, Indonesia, 20123

Abstract. Metamizole is a nonsteroidal antiinflammatory drug (NSAID) that functions as an analgesic, antipyretic, and antiinflammatory. Examination of active substance contents is a requirement that must be met to ensure the quality of drug preparations. The aims of this study were to develop and validate the Fourier Transform Infrared spectrophotometric method for the quantitation of metamizole content in marketed tablet preparation. Identification and determination of metamizole contents by Fourier Transform Infrared spectrophotometric method used methanol solvent in the wavenumber range 4000 cm⁻¹ to 650 cm⁻¹. The results showed that the specific wavenumbers of metamizole were 1649.3 cm⁻¹; 1623.3 cm⁻¹; and 1589.7 cm⁻¹; and the contents metamizole in marketed tablet preparation ranged from (97.954 ± 0.121)% to (104.541 ± 0.257)%. From the validation method, the recovery result is 100.129%; the relative standard deviation is 0.057%; the limit of detection is 2.09526 mg/mL; the limit of quantitation is 6.34928 mg/mL; and the range 40 mg/mL to 60 mg/mL. The quantitation of metamizole contents can be carried out by Fourier Transform Infrared spectrophotometric method with accurate and precise quantitation results.

Keywords: Fourier Transform Infrared, spectrophotometric, identification, determination, metamizole

INTRODUCTION

Metamizole is methanesulfonate derivate from aminopyrine which acts on the central nervous system to reduce the sensitivity of pain receptors and affect central body temperature control [1]. Metamizole has three main effects as a drug: analgesic, antipyretic, and antiinflammatory. Analgesic drugs relieve pain by increasing the pain threshold value in the central nervous system without suppressing consciousness. Antipyretic drugs reduce high body temperature. Antiinflammatory helps overcome inflammation [2].

It is absolutely necessary to inspect the quality of the preparation of a drug to ensure that the pharmaceutical preparation uses ingredients with standard quality and quantity with suitable analytical procedures to support the expected therapeutic effect. In order for the quality of the drug to be guaranteed and effective in treatment, it is necessary to have an appropriate content of active substances involved in the drug preparation [3,4].

The Fourier Transform Infrared spectrophotometric method is a very popular analytical technique for the analysis of various types of samples, whether samples are pharmaceutical products, food, biological fluids, or environmental samples [5].
Quantitative analysis of the components in the solution can be carried out successfully by the Fourier Transform Infrared spectrophotometric method, providing a suitable band in the spectrum of the target component (analyte). The bands selected should have high molar absorptivity values, not overlap with solvent peaks, not overlap with other components in a mixture, and provide a linear calibration plot [6].

The Fourier Transform Infrared spectrophotometric method is an excellent technique for pharmaceutical analysis and offers many advantages since it is easy to use, sensitive, selective, environmentally friendly, and fast. It also helps ensure regulatory compliance through validation protocols [7]. Supervision of metamizole tablet preparation needs to be maintained because if they do not meet the requirements it can endanger consumers. The Fourier Transform Infrared spectrophotometric method was used to determine the contents of metamizole in this study. The aims of this paper are the development and validation of the Fourier Transform Infrared spectrophotometric method for the quantitation of metamizole content in marketed tablet preparation in a simpler, cheaper, faster, and more environmentally friendly way.

**METHODODOLOGY**

**Types of research**

This research paper is a descriptive study using Fourier Transform Infrared spectrophotometric method to quantify metamizole contents in marketed tablet preparation.

**Tools**

The tools used in this study were Fourier Transform Infrared spectrophotometer Cary 630 with Dial Path (Agilent), a light source, interferometer and detector, computer (Lenovo), printer (Epson), mortar and pestle (Shimadzu), analytical balance (Sartorius), filter paper (Whatman), tissue (Paseo), multipipette (Eppendorf), sonicator (Krisbow), volumetric flask (Iwaki), and other glassware (Iwaki).

**Materials**

The materials used in this study were methanol (Merck), metamizole working standard (Mutiara Mukti Farma), and marketed metamizole tablets: Novalgin® (Aventis Pharma), Selesgin® (Sejahtera Lestari Farma), Licogin® (Berlico Mulia Farma), Unigin® (Universal Pharmaceutical Industries), Etalgoin® (Errita Pharma), Metamizole (Kimia Farma), Metamizole (Corsa Industries), Metamizole (Holi Pharma).

**Sampling**

The sampling technique used was a combination of purposive sampling and randomized sampling.

**Stock Solution**

Five g of metamizole was weighed, inserted into a 50 mL volumetric flask, and 30 mL of methanol was added. It was then sonicated for 30 minutes, methanol was added to the marked line, and the solution was shaken until homogenously mixed (concentration of 100 mg/mL).

**Metamizole Vibration Spectrum**

Five mL of stock solution was pipetted and inserted into a 10 mL volumetric flask. Then, methanol was added to the marked line, and shaken until homogenously mixed (concentration of 50 mg/mL). A standard solution and methanol (blank) vibration spectrum was measured with 0.1 mm cell at wavenumber 4000 cm⁻¹ to 650 cm⁻¹, and the specific wavenumbers of metamizole were analyzed.

**Calibration Curve**

The standard metamizole solution is made in 10 mL volumetric flask with concentration of 30 mg/mL to 70 mg/mL. Then, the vibration spectrum is measured with a 0.1 mm cell at wavenumber 4000 cm⁻¹ to 650 cm⁻¹, and then the following were calculated: the regression equation, determination coefficient, and correlation coefficient by used the obtained area.

**Quantitation of Metamizole**

Quantitation method used in this study was modified from Robaina et al. (2013). The 20 tablets for each sample (brand name or generic name) were weighed andpowdered, then weighed powders equivalent to 500 mg of metamizole were inserted into a 10 mL volumetric flask and 6 mL of methanol was added. The solution was sonicated for 30 minutes, and then methanol was added to the marked line. It was shaken until homogenously mixed, then the mixture was filtered. After that, 1 mL of the first filtrate was removed, and the next filtrate were collected (theoretical concentration 50 mg/mL). The peak area was measured, the obtained concentration was calculated, and the metamizole content in the sample was calculated as well.

**Validation**

The validation method used in this study was modified from Maggadani et al. (2020). Validation was carried out using parameters of accuracy (recovery percentage), precision (relative standard deviation), linearity (correlation coefficient), the limit of detection,
the limit of quantitation, range, and specificity. The accuracy test was carried out by measuring the percentage of recovery in three specific ranges, (80%, 100%, and 120%), wherein each specific range used 70% of the samples analyzed and 30% of the standard added (standard addition method). The precision test was carried out by calculating the relative standard deviation of the percentage of recovery data obtained from accuracy test [9].

RESULTS AND DISCUSSION

Metamizole Vibration Spectrum

This study started with determining the vibration spectrum of methanol. The methanol vibration spectrum can be seen in Figure 1.

Figure 1. Methanol vibration spectrum (Above is full spectrum. Below is zoom spectrum.)

The study continued by determining the vibration spectrum of metamizole 50 mg/mL in methanol. Metamizole vibration spectrum can be seen in Figure 2.

Figure 2. Metamizole vibration spectrum (Above is full spectrum. Below is zoom spectrum.)

The overlap of the metamizole vibration spectrum and methanol vibration spectrum can be seen in Figure 3.

Figure 3. Overlap of the metamizole vibration spectrum and methanol vibration spectrum (Metamizole - Red Line; Methanol - Blue Line) (Above is full spectrum. Below is zoom spectrum.)

Overlapping was used to determine the specific wavenumber of metamizole vibration spectrum. An overlap of the metamizole vibration spectrum and methanol vibration spectrum can be seen in Figure 3.

A significant difference in the spectrum can be seen between the wavenumbers 1500 cm\(^{-1}\) to 1700 cm\(^{-1}\), at 1649.3 cm\(^{-1}\); 1623.3 cm\(^{-1}\); and 1589.7 cm\(^{-1}\). The wavenumbers is specifically indicative of metamizole and not indicative of methanol, so that a qualitative analysis and a quantitative analysis can be carried out by specific wavenumber region of metamizole [10]. Each molecule has a unique infrared spectrum, so that it can be distinguished from other molecules by the position and intensity of the absorption band [11].

The Fourier Transform Infrared spectrophotometric method is known as the fingerprint profile because of its ability to distinguish all evaluated samples. The Fourier Transform Infrared spectrophotometric method is also an ideal technique because it is simpler, cheaper, faster, and more environmentally friendly [12]. The Fourier Transform Infrared spectrophotometric method is an excellent analytical technique in the process of identifying the molecular structure of a compound. The infrared spectrum can show the absorption of each bond in a molecule so that each molecule or compound has a different spectrum pattern [13].

The Fourier Transform Infrared spectrophotometric method does not require complicated sample preparation and can be used in various phases: solid, liquid and gas.
The absorption spectrophotometric method used is based on differences in the absorption of infrared radiation by the molecules of a material. The infrared absorption by a material can occur if two conditions are met, namely the conformity between the infrared radiation frequency and the vibrational frequency of the sample molecules and the change in the dipole moment during vibration [14].

**Calibration Curve Results**

The metamizole vibration spectrum with various concentrations with calibration curve series can be seen in Figure 4.

![Figure 4. Metamizole vibration spectrum at various concentrations range from 0 mg/mL to 80 mg/mL. (Above is full spectrum. Below is zoom spectrum).](image)

The Fourier Transform Infrared spectrophotometric method has advantages such as qualitative analysis, where each molecule will certainly provide a different absorption spectrum [15]. According to research result, the quantitation of contents was carried out by the method of specific spectrum area.

The vibrational spectrum of metamizole at various concentrations in methanol shows that the concentration does not change the shape of the spectrum at specific wavenumbers but only changes the absorption intensity of each concentration. Thus, it can be said that the use of methanol as a solvent is stable for metamizole analysis. From the figure above, it can be seen that higher concentrations mean greater absorption intensity, and greater heights mean greater areas.

The standard metamizole calibration curve is made by plotting the concentration (X) with the area (Y), then the points are connected by a straight line. The metamizole regression equation, determination coefficient \( r^2 \), and correlation coefficient \( r \) were calculated. The metamizole calibration curve can be seen in Figure 5.

Based on the curve above, the metamizole regression equation obtained was \( Y = 0.7883743 \times X + 30.5865533 \); the determination coefficient \( r^2 \) was 0.99517; the correlation coefficient \( r \) was 0.99758. The value of determination coefficient \( r^2 \) is not less than 0.997 and the value of correlation coefficient \( r \) is more than 0.990, which indicates that the area (Y) was well determined and well correlated with the concentration (X) [16].

**Identification and Determination of Metamizole Results**

The qualitative analysis (identification) and quantitative analysis (determination) of metamizole contents was carried out using the Fourier Transform Infrared spectrophotometric method. The qualitative analysis was carried out by detection of the specific peak metamizole on wavenumber 1649.3 cm\(^{-1}\), 1623.3 cm\(^{-1}\), and 1589.7 cm\(^{-1}\). The concentration of metamizole in the sample was determined based on the regression equation of the calibration curve from the standard.

The marketed metamizole tablet preparation with both brand and generic names were determined based on area, all in accordance with the general requirements of active pharmaceutical ingredient content in tablet preparation (not less than 90.0% and not more than 110.0% of the amount stated on the label) [17].
Development of Fourier Transform Infrared Spectrophotometric Method for Quantitation of Marketed Metamizole Tablet
(Nerdy Nerdy, Effendy De Lax Putra, Jihan Safira)

Table 3. Metamizole contents in tablet preparation

| Sample                  | Contents                      |
|-------------------------|-------------------------------|
| Novalgin®               | (104.541 ± 0.257)%            |
| Selesgin®               | (103.812 ± 0.231)%            |
| Licogin®                | (99.323 ± 0.180)%             |
| Unigin®                 | (102.457 ± 0.215)%            |
| Etalgin®                | (101.702 ± 0.205)%            |
| Metamizole (Kimia Farma)| (97.954 ± 0.121)%             |
| Metamizole (Corsa Industries)| (98.918 ± 0.139)%          |
| Metamizole (Holi Pharma)| (100.203 ± 0.193)%           |

Note: samples with ® means marketed samples with a brand name, and samples without ® means marketed samples with a generic name.

Validation Results
The validation parameters tested were accuracy, precision, linearity, detection limit, quantitation limit, and range. The accuracy test is expressed in the recovery percentage determined by the standard addition method. The precision test is expressed in relative standard deviation obtained from the accuracy test. The validation test was carried out with the standard addition method on a sample with content closest to 100.0% (sample Z).

The average recovery percentage, 100.129%, was declared to meet the accuracy requirements because method validation is in the range of 98% to 102%. The method of analysis of metamizole content was stated to be accurate. The relative standard deviation was 0.057%. This result fulfilled the precision requirements for method validation with the value of less than 2% [18,19].

The linearity obtained from the calibration curve resulted in a correlation coefficient of 0.99758 and was declared to meet the linearity requirements for method validation because the value not less than 0.995 [20]. The limit of detection and the limit of quantitation were calculated from the calibration curve. The limit of detection for metamizole is 2.09526 mg/mL and the limit of quantitation for metamizole is 6.34928 mg/mL. The range for metamizole quantitation by Fourier Transform Infrared spectrophotometric method is in the range 40 mg/mL (80%) to 60 mg/mL (120%). This method shows good specificity to detect metamizole in various metamizole tablet matrices for qualitative analysis and quantitative analysis.

CONCLUSION
The identification and determination process of metamizole content in various types of tablets can be carried out in a simpler, cheaper, faster, and more environmentally friendly way by using the Fourier Transform Infrared spectrophotometric method with methanol as a solvent. The metamizole contents in all marketed metamizole tablets with a brand name or generic name met the general requirements of active pharmaceutical ingredient content in tablets preparations (not less than 90.0% and not more than 110.0% of the amount stated on the label).

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest in this research and this article.

ACKNOWLEDGEMENTS
The authors would like to thank the Institut Kesehatan Deli Husada Deli Tua for research funding. The authors also would like to thank the Mutiara Mukti Farma Industri Farmasi for research tools and materials support.

REFERENCES
[1] Miljkovic, M.; Dragojevic-Simic, V.; Rancic, N.; Simic, R.; Pekez-Pavlisko, T.; Kovacevic, A.; and Stamenkovic, D. 2018. Metamizole Utilization and Expenditure During 6 Year Period - Serbia versus Croatia. Front. Public Health. 6 213.
[2] Cacau, I.; Mogosan, C.; and Loghin, F. 2015. Safety Issues of Current Analgesics - an Update. Clujul Med. 88(2) 128-136.
[3] Gouveia, B.G; Rijo, P.; Gonçalo, T.S.; and Reis, C.P. 2015. Good Manufacturing Practices for Medicinal Products for Human Use. J. Pharm. Bioallied Sci. 7(2) 87-96.
[4] Taylor, D. 2015. The Pharmaceutical Industry and the Future of Drug Development. Pharmaceuticals in the Environment. 1st Ed. Vol 41. (Cambridge: RSC Publisher) 1-33.
[5] Baker, M.J.; Trevisan, J.; Bassan, P.; Bhargava, R.; Butler, H.J.; Dorling, K.M.; Fielden, P.R.; Fogarty, S.W.; Fullwood, N.J.; Heys, K.A.; Hughes, C.; Lasch, P.; Martin-Hirsch, P.L.; Obinaju, B.; Sockalingum, G.D.; Sulé-Suso, J.; Strong, R.J.; Walsh, M.J.; Wood, B.R.; Gardner, P.; and Martin, F.L. 2014. Using Fourier transform IR Spectroscopy to Analyze
Development of Fourier Transform Infrared Spectrophotometric Method for Quantitation of Marketed Metamizole Tablet
(Nerdy Nerdy, Effeny De Las Putra, Jihan Safira)

[98x799]Biological Materials. Nat. Protoc. 9(8) 1771-1791.
[6] Fritzschke, A.; Ritschel, T.; Schneider, L.; and Totsche, K.U. 2019. Identification and Quantification of Single Constituents in Groundwater with Fourier Transform Infrared Spectroscopy and Positive Matrix Factorization. Vib. Spec. 100 152-158.
[7] Ouhaddouch, H.; Cheikh, A.; Idrissi, M.O.B.; Draoui, M.; and Bouatia, M. 2019. FTIR Spectroscopy Applied for Identification of a Mineral Drug Substance in Drug Products: Application to Bentonite. J. Spec. 2019 290845
[8] Robaina, N.F.; de Paula, C.E.R.; Brum, D.M.; de la Guardia, M.; Garrigues, S.; Cassella, R.J. 2013. Novel Approach for the Determination of Azithromycin in Pharmaceutical Formulation by Fourier Transform Infrared Spectrophotometric in Film through Transmission Mode. Microchem. J. 110 (2013): 301-307.
[9] Maggadani, B.; Oktaviani, E.; Harahap, Y.; and Harmita, H. 2020. Optimization and Validation of an Analytical Method for Tranexamic Acid in Whitening Creams by RP HPLC with Precolumn Derivatization. Int. J. App. Pharm. 12(1) 167-171.
[10] Moffat, A.C.; Osselton, M.D.; and Widdop, B. 2011. Clarke’s Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material. 4th Ed. (Noida: Pharmaceutical Press) 1279-1280.
[11] Balan, V.; Mihaic, C-T.; Cojocaru, F-D.; Uritu, C-M.; Dodi, G.; Botezat, D.; and Gardikiotis, I. 2019. Vibrational Spectroscopy Fingerprinting in Medicine: from Molecular to Clinical Practice. Materials. 12 2884
[12] Fanelli, S.; Zimmermann, A.; Toto’li, E.G.; and Salgado, H.R.N. 2018. FTIR Spectrophotometry as a Green Tool for Quantitative Analysis of Drugs: Practical Application to Amoxicillin. J. Chem. 2018 3920810
[13] da Silva, H.R.G.; Quinella C.M.; and Meira, M. 2017. Separation and Identification of Functional Groups of Molecules Responsible for Fluorescence of Biodiesel Using FTIR Spectroscopy and Principal Component Analysis. J. Braz. Chem. Soc. 28(12) 2348-2356.
[14] Kumirska, J.; Czerwicka, M.; Kaczyński, Z.; Bychowska, A.; Brzozowski, K.; Thömig, J.; and Stepnowski, P. 2010. Application of Spectroscopic Methods for Structural Analysis of Chitin and Chitosan. Mar. Drugs. 8(5), 1567-1636
[15] Rohman, A. 2012. Application of Fourier Transform Infrared Spectroscopy for Quality Control of Pharmaceutical Products - A Review. Indonesian J. Pharm. 23(1) 1-8
[16] Asuero, A.G.; Sayago, A.; and Gonzalez, A.G. 2006. The Correlation Coefficient - An Overview. Critical Reviews in Analytical Chemistry, 36:41-59.
[17] Veronin, M.A.; Nutan, M.T.; and Dodla, U.K.R. 2014. Quantification of Active Pharmaceutical Ingredient and Impurities in Sildenafil Citrate obtained from the Internet. Ther. Adv. Drug Saf. 5(5) 180-189
[18] Le, T.H.H.; Phung, T.H.; and Le, D.C. 2019. Development and Validation of an HPLC Method for Simultaneous Assay of Potassium Guaiacolsulfonate and Sodium Benzoate in Pediatric Oral Powder. J. Anal. Methods Chem. 2019 6143061
[19] Le, D.C.; Ngo, T.D.; and Le, T.H.H. 2019. Simultaneous Assay of Dextchlorpheniramine Maleate, Betamethasone, and Sodium Benzoate in Syrup by a Reliable and Robust HPLC Method. J. Anal. Methods Chem. 2019 2952075
[20] Setyawati, A. 2019. Analysis Methods Verification of Boron in River Water Using the UV-Vis Spectrophotometer with Curcumin Complex as Alternative Practical Educations. Int. J. Chem. Ed. Res. 3(2) 60-65.