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Development of ivermectin and ivermectin tablet monographs for the International Pharmacopoeia

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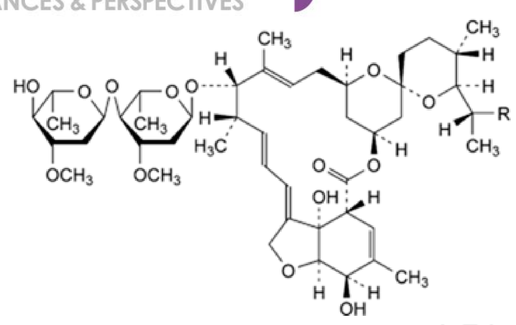

Component	R	Molecular formula
H_2B_{1a}	CH_2-CH_3	$C_{48}H_{74}O_{14}$
H_2B_{1b}	CH_3	$C_{47}H_{72}O_{14}$

Abstract

During the process of developing a monograph for the International Pharmacopoeia (IntPh), a transparent process and a broad public consultation must be warranted so that the texts that are approved are made public accurately.
After each meeting of the WHO-Expert Committee on Pharmaceutical Preparations and subject to the availability of resources, the Secretariat of the Committee seeks to publish monographs that have been adopted for inclusion in the IntPh.
The monographs that appear in the IntPh are an important element of the quality of medicines (including safety and efficacy) that appear on the WHO List of Essential Medicines.
Ivermectin, an anti-parasitic semi-synthetic macrolide agent is used in public health campaigns, and the quality of the medicines distributed depends on the existence in the Pharmacopoeia of validated methods and appropriate quality standards. The WHO-Expert Committee on Specifications for Pharmaceutical Preparations considers a priority to include in the IntPh active pharmaceutical ingredients and finished pharmaceutical products for high prevalence tropical diseases in low income populations such as helminthiasis, human lymphatic filariasis, strongyloididasis, onchocerciasis, scabies, etc.
Based on the scientific literature search and related standards that appears in other pharmacopoeias, methods of analysis and quality specifications have been proposed that must be checked / verified or experimentally modified to present the laboratory reports for consideration by the Committee of Experts for possible approval and inclusion in the International Pharmacopoeia. The work seeks to demonstrate that the proposed methods are suitable for acceptance of inclusion in the International Pharmacopoeia as monographs. Therefore, the preliminary results of these 2 new-monographs are presented herein.

Requirements

Definition. Ivermectin contains not less than 95.0% and not more than 102.0% of the sum of the Ivermectin components H_2B_{1a} ($C_{48}H_{74}O_{14}$) and H_2B_{1b} ($C_{47}H_{72}O_{14}$), calculated with reference to the anhydrous and solvent-free substance. The ratio of the area percentages of component H_2B_{1a} / (H_2B_{1a} + H_2B_{1b}) is not less than 90.0%.

Methodology and Results

Test ivermectin	*Method*	*Experimental conditions*	*Materials and equipment*	*Quality Specifications*	*Results*
Identity	IR	KBr discs, scan number : 20, Resolution: 4cm^{-1}, Apodization: Happ-Genzel	Spectrophotometer FT-IR, Shimadzu, Mod. IR Affinity-1 Agathe Mortar & pestle, hydraulic press 400-4000	The infrared absorption spectrum is concordant with the spectrum obtained from ivermectin RS or with the reference spectrum of ivermectin.	IR spectra of ivermectin API concordant with spectra from RS or Rspec
	HPLC	See Assay Section	See Assay Section 1.14.4 HPLC IntPh(2016)	Chromatograms: Main peaks Rt of Sol(1) = Rt of Sol(2)	Rt's are corresponding
	TLC	MobPhase: CH_2CL_2(90)/MeOH(9)/ NH_3(1)	IntPh(2016). TLC 1.14.1 Plates 20cmx20cm Silica gel GF_{254} o HF_{254}	The 2 partly-separated spots with solution (A) correspond in position, appearance, and intensity with correspondent spots due to ivermectin in the chromatogram obtained with solution (B).	Rfs, appearance and intensity are corresponding
Solubility	Qualitative	Room temperature	Dehydrated alcohol R/dichloromethane R/dH_2O	Freely soluble/soluble/practically insoluble	API analysed complies
Heavy Metals	Qualitative	As per IntPh (2016): 2.2.3 Limit test for Heavy Metals, Method A	Nessler tubes, dimensions: see IntPh (2016)	NMT 20µg/g	API analysed complies
Sulfated Ash(2.3)	Method A	IntPh (2016). 2. Method of Analysis 2.3 Sulphated Ashes;1g	Muffle Naabertherm L9/11/S2, Platinum crucible, balance Mettler Toledo New Classic Mod ML204	NMT 3mg/g	API analysed complies
Clarity/Color of solutions	Qualitative	IntPh(2016), *1.11.2 Degree of coloration of liquids*, Method II.	Reagents	Solutions is clear and not more intensely coloured than Reference Solution BY_7	API analysed complies
Water	Karl-Fisher, Method A	0.500g-1.000g of substance; Dihidrate sodium tartrate, HYDRANAL (Fluka) KF reagetnt	Automatic KF titrator, Mod DL31, Mettler Toledo	NMT 10mg/g (1%)	API analysed complies
Specific Optical Rot	Polarimetry	Conc. 2.5036g/100mL in 25 mL MeOH	Automatic Polarimeter WZZ-3, tube length 100mm; T=20°C	$[\alpha]_D^{20} = -20$ to -17 with reference to the anhydrous and solvent-free substance	API analysed complies
Related Substances	HPLC	Inject 20 µL of each: Sol(1): 800µg/mL- Sol(2): 8mγ/mL Sol(3): 0.4µg/Ml	See HPLC conditions under Assay	Sol(1): RRT H_2B_{1b}/H_2B_{1a} ~0.74; Resolution between H_2B_{1b} & H_2B_{1a} is at least 3; SNR at least 10 Sol(2): Area of any impurity peak with RRT 1.3-1.5 NGT 2.5%; Area of any impurity NGT area of main peak Sol(3): Σ of all areas of impurity peaks NGT 5 times the area of principal peak, 5%; Disregard any peak with an area less than the area of the principal peak in the chromatogram obtained	API analysed complies
Ethanol and Formamide	IntPh(16) GLC Method 1.14.5	LinGrad(0-2min) 50°-80°C/(2-8min)80°240°C; Detec 250ºC Press 6.31psi; IP 220°C T Injection port 220ºC;Rel1:10 Injection vol 1µl.	Gas Chormatograph Agilent Model 7890A Metler Toledo Balance New Classic Mod. ML204. Column: Agilent DB 624 30m x 530µm ID y 0.3µm, 260ºC max temperature	NMT 50 mg/g Ethanol; NMT 30 mg/g Formamide	API analysed complies
Assay	HPLC Shimadzu Prominen/ LabSolutioSoftware	Method EuPh/Merck Mobile Phase dH_2O/MeOH/MeCN, Column RESTEK C18, 25cm Sol(1) API 0.8mg/mL(10µL) Sol(2) RS 0.8 mg/mL(10µL)	Definition: Ivermectin contains NLT 95.0% and NMT 102.0% of sum of the Ivermectin H_2B_{1a} and H_2B_{1b}, calculated with reference to the anhydrous and solv-free substance. The ratio of the area percentages of component H_2B_{1a} / (H_2B_{1a} + H_2B_{1b}) is not less than 90.0%.	Sol(2): RRT H_2B_{1b}/H_2B_{1a} ~0.74; Resolution between H_2B_{1b} & H_2B_{1a} is at least 3 From chromatog of Sol(1)&Sol(2) calculate %content of ivermectin(H_2B_{1a}+H_2B_{1b}) considering the assigned contents of component H_2B_{1a} and component H_2B_{1b} in ivermectin RS, and the ratio H_2B_{1a}/ (H_2B_{1a} + H_2B_{1b})	API analysed complies

Test ivermectin tablets	Methods	Equipment/conditions	Materials/Methods	Quality Spefications	Results
Identity	HPLC	See under Assay	See under Assay	Rt main peaks in chromatogram solution (1) = RT peaks due to ivermectin in chromatogram obtained solution (2).	Tablets analysed complies
	TLC	IntPh(16), 1.14.1 TLC. Mobile phase: CH_2CL_2(90)/MeOH(8)/ NH_3(0.8). Apply 30µL of Sol(A) tab extract and Sol(B) RS	Plates 20cmx20cm Silica gel GF_{254} o HF_{254},	The 2 partly separated main spots in the chromatogram obtained with solution (A) correspond in position, appearance, and intensity with the two partly separated principal spots due to ivermectin in the chromatogram obtained with solution (B)	Tablets analysed complies
Disolution test	Disolution apparatus/ HPLC; IntPh(16) Dissol. Test for SDF	900mL, 0.5% sodium dodecyl sulfate in phosphate buffer, pH7.0(0.01mol/L; paddle 50rpm; 45min; filtered sample 10.0mL Sol(1);Standard solution 33.0mg ivermectine/250mL medium; Sol(3) (3mg sample):5mL Sol(2)/200mL; Sol(3) 6mg sample):5mL Sol(2)/100mL	Disolution apparatus, HPLC, RS, buffer, paddle, ivermectin tablets 3mg and 6mg; inject 100 µL of each Sol(1) and Sol(3); Tablet samples:mectizan 3mg, Ivertex 6mg, Ivermectin Calox 6 mg	For each of the tablets calculate the total amount of ivermectin (component H_2B_{1a} and component H_2B_{1b}) in the medium considering the assigned contents of component H_2B_{1a} and component H_2B_{1b} in ivermectin RS. Evaluate the results as described under *5.5 Dissolution test for solid oral dosage forms, Acceptance criteria.* The amount of ivermectin (component H_2B_{1a} and component H_2B_{1b}) in solution for each tablet is not less than 80% (Q) of the amount declared on the label	3mg and 6 mg (Ivertex 6mg, Calox 6mg, mectizan 3mg) tablets analysed complies with dissolution specs
Assay	HPLC	Mobile phase dH_2O(12)/MeOH(35)/MeCN(53); 1.2mL/min; Column RESTEK C18, 25cm; UV detector, 245nm; Sol(1) content of 25mg equiv /100mL;Sol(2) 25mg/100mL; inject 10µL	USP/EurPh/Merck methods. Shimadzu Prominen/ LabSolutioSoftware HPLC: IntPh(16) 1.14.4; Stainless-steel column, 25cm, C18.	Measure the areas of the peaks corresponding to the components H_2B_{1a} and H_2B_{1b} obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of ivermectin (component H_2B_{1a} and component H_2B_{1b}) in the tablets considering the assigned contents of component H_2B_{1a} and component H_2B_{1b} in ivermectin RS	3mg and 6 mg (Ivertex 6mg, Calox 6mg, mectizan 3mg) tablets analysed complies with dissolution specs
Uniformity of content	HPLC	For Sol(1) (3mg/6mg tabs) content of 1tab/25mL For 6mg tabs dilute 5.0mL of Sol(1) to 10.0mL. For Sol(2) 30.0mg RS / 250.0mL. Inject 25µL of each Sol(1) and Sol(2)	USP/EurPh/Merck methods. Shimadzu Prominen/ LabSolutioSoftware HPLC: IntPh(16) 1.14.4; Stainless-steel column, 25cm, C18. Reference standard ivermectin in glycerol formal and SQR, methanol	Measure the areas of the peaks corresponding to the components H_2B_{1a} and H_2B_{1b} obtained in the chromatograms of solution (1) and (2) and calculate the percentage content of ivermectin (component H_2B_{1a} and component H_2B_{1b}) in each tablet using the declared contents of component H_2B_{1a} and component H_2B_{1b} in ivermectin RS	3mg and 6 mg (Ivertex 6mg, Calox 6mg, mectizan 3mg) tablets analysed complies with assay specs
Related substances	HPLC	Mob ph water R/ MeOH R /AcCN R(39/55/106). 1.5 mL/min. Detector UVSpec cell, lmáx at 245 nm. Impurity D, λmáx 280nm Sol(1) 25mg Equiv ivermectin/100mL; Sol(2) 1ml Sol(1)/100mL; Sol(3) 25mg RS/50mL; Sol(4) 1mL Sol(3)/100; Sol(5) 5.0 µg of 3-*tert*-butyl-4-hydroxyanisole R /mL; Sol(6) CuBr soln.	Carry out the test as described under PhInt *1.14.4 HPLC* using an SS column (25cm × 4.6mm) packed with silica gel, surface modified with chemically-bonded octadecylsilyl groups (5 µm). (A Restek or Agilent PoroShell column or a Zorbax SB C18 column were found suitable). Shimadzu Prominent/Labsolutionsoftware. In situ oxidations process: Add 5.0 mL of solution (1) and 100 µL of tert-butyl hydroperoxide R and dilute to volume. Mix well and let the solution stand at room temperature for approximately 20 minutes. Inject 20mL of each solutions (1)-(6).	Use chromatograms obtained to determine compliance • Area peak area from impurity D (28-oxoH_2B_{1a}) NGT area main peak in chromatogram from Sol (5) (2.0%); • Area any impurity peak with a RRT 1.3-1.5 ref main peak NGT 2.7 times area main peak from SoL (2) (2.7%); • Area any impurity peak NGT area of main peak from solution(2) (1%); • Sum of areas all impurities, other than impurity D, with Solution (2) as a reference solution. Disregard any peak with area less than 0.1 times area of main peak from Sol(2) (0.1%). Calculate sum of impurities considering concentration found for impurity D. The sum of all impurities is NGT 6%.	3mg and 6 mg (Ivertex 6mg, Calox 6mg, mectizan 3mg) tablets analysed complies with assay specs

Conclusions.
It has been possible to demonstrate that the methodological conditions that were proposed are reproducible with accuracy. Additionally, having managed to analyze 3 commercial products using the proposed methods demonstrates the robustness of the proposed methodology, an important aspect to fulfill for the monographs of the International Pharmacopoeia

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